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# Pharmacodynamic Evaluation of the Additive Combination of Pterostilbene and Oxacillin against Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33591

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## Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) was initially limited to hospital and healthcare facilities but has gradually become a growing problem in healthy children and adult. Pterostilbene belongs to the phenylpropanoid phytoalexin which is involved in plant response to various pathogen and herbivores attack. The aim of this study was to evaluate the anti-MRSA action of pterostilbene in combination with selected antibiotics such as vancomycin, linezolid and oxacillin against MRSA ATCC 33591. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of individual antimicrobial agents were determined using microbroth dilution technique whereas the microdilution checkerboard (MDC) assay was employed to verify the type of interaction of the combined agents from the fractional inhibitory concentration (FIC) index values. Time- kill assay (TKA) analysis and post-antibiotic effect (PAE) time were determined only on the combination which showed synergistic interaction. Cell morphology and ultrastructural changes of the treated and untreated strains were also observed. The MIC and MBC of pterostilbene against ATCC 33591 were 31.25μg/ml and 62.50μg/ml, respectively. This indicated that pterostilbene was bacteriostatic against ATCC 33591. MDC results showed that pterostilbene-oxacillin combination exhibited lowest FIC value (0.56) for ATCC 33591 which implies partial synergistic interaction. On the other hand, combination of pterostilbene and vancomycin generated an additive effect (FIC 1.00) whereas pterostilbene-linezolid combination displayed indifference effects with FIC of 1.25 against MRSA ATCC 33591. Despite the partial synergism, TKA proved an additive effect for the combination of pterostilbene and oxacillin against ATCC 33591 with concentration-dependent bactericidal action within 24 hour. After one hour exposure at 10X-MIC, prolonged PAE time of 2.6 ± 1.48 hour against ATCC 33591 was demonstrated by pterostilbene-oxacillin combination treatment compared to pterostilbene (2.02 ± 0.36 hour) and oxacillin (0.53 ± 0.28 hour) alone. Scanning and transmission electron microscopic observations revealed that pterostilbene targeted the cell wall which is the same site of action as oxacillin hence additive effects by the combination treatment. In conclusion, pterostilbene in combination with oxacillin showed partial synergism with bactericidal and persistent antimicrobial effect against MRSA ATCC 33591. Therefore, pterostilbene has the potential to be developed as an alternative phytotherapeutic agent against MRSA infections.

**Keywords**: Pterostilbene, MRSA, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), Fractional Inhibitory Concentration (FIC), Bactericidal, Time-Kill Assay (TKA), Post-Antibiotic Effect (PAE).

## 1. Introduction

*Staphylococcus aureus* is a normal microflora that can be found in the skin and nose of healthy people. However, these bacteria will become opportunistic pathogens when these bacteria enter the human body through small surgical wound or trauma on someone who has weakened immune system causing skin and soft tissue infections [1]. Infections caused by *S. aureus* have been treated with various antibiotics such as penicillin, macrolides and aminoglycosides. However, these bacteria have managed to grow and resistant against various type of antibiotic [2]. The emergence of MRSA not only give special attention because of resistance against methicillin but also due to resistant against almost all kinds of existing antibiotics even though there are new antibacterial drugs on the market [3,4]. These bacteria caused many infections in communities such as endemic and epidemic nosocomial infections [5].

Therefore, to find ways and strategies to prevent or delay the development of resistance of MRSA, various studies have been conducted to find an alternative treatment to overcome this phenomenon from becoming more serious. The step is to use a combination of antimicrobial therapy that consists of existing antibiotics and plant extracts [6]. Natural products and their derivatives have been identified over the years as a source of therapeutic agent [7,8]. Natural products derived from plants, animals and minerals has been the basis for the treatment of humans since long ago [9,10,11]. Natural product is an organic or chemical compounds produced by living organisms in nature that usually have a pharmacological or biological activity that are valuable and can be used in the invention and design of drugs [12].

Phytochemicals are bioactive compounds derived from plants and the term is often used to describe a large number of secondary metabolites [13]. Pterostilbene is a component that belongs to a phenolic group known as stilbene [14]. Stilbene was secondary metabolites of the plants that formed from flavonoid biosynthesis pathway and belongs to the family of phenylpropanoid [15]. Pterostilbene found in sandalwood *Pterocarpus santalinus* by [16], hardwood of *Pterocarpus marsupium* by [17], leaves of *Vitis vinifera* by [18] and also found in some species of *Vaccinium* berries [19]. Pterostilbene also is an analog of resveratrol found in grapes and blueberries [20]. This component is biologically classified as a phytoalexin. Antimicrobial phytoalexin is a plants defense system which is involved in plant response to various pathogen and herbivores attack [15].

In addition, antibiotics used in this study were vancomycin, oxacillin and linezolid. Vancomycin and oxacillin known as agents that target cell wall while lonezolid targeting protein synthesis [21,22,23]. Evaluation on the morphology and ultrastructure of cells after treatment studies using electron microscopy. Transmission electron microscope (TEM) is typically used by microbiologists to study on intracellular or cell wall structure [24,25,26,27]. Scanning electron microscope (SEM) was used to study the surface of the cell and its features [28,29,30,31].

## 2. Results and Discussion

### *2.1 Determination of MIC and MBC value.*

MIC and MBC of pterostilbene for MRSA ATCC 33591 was 31.25 µg / ml and 62.50 µg / ml (Table 1). The MBC is two-fold higher than the MIC for both strains. Therefore, this indicated that pterostilbene has potential as an antimicrobial agent that produce bacteriostatic effects. MIC and MBC values of oxacillin against ATCC 33591 was same, each value 62.50 µg/ ml (Table 2). Therefore, oxacillin exhibit antimicrobial bactericidal agent. The MIC and MBC of vancomycin against ATCC 33591 was same, 0.98µg / ml (Table 3). Therefore, this suggests that vancomycin exhibits bactericidal activity

against ATCC 33591. MIC values of linezolid against MRSA ATCC 33591 was 1.56 µg / ml (Table 4). MBC values for linezolid shows that linezolid displayed bacteriostatic activity against MRSA strain which is four times higher than the MIC of ATCC 33591.

This study showed that pterostilbene exhibit stronger antibacterial properties than oxacillin on MRSA ATCC 33591. Pterostilbene is a phytoalexin which belongs to the phenylpropanoid family that involved in plant response to various pathogens and herbivores attack [15]. Comparison with antibiotics that are used in this study as current therapy against MRSA infection, suggesting that the antimicrobial activity of pterostilbene is lower than linezolid and vancomycin on ATCC 33591. It is supported by [32] which states that phytochemicals produce lower activity compared to standard antibiotics.

Pterostilbene may be able to reduce the problem of microbial resistance that was supported by [33] reported that phytochemicals capable of exhibiting significant potential for changing the resistivity of an antibiotic. Although the antimicrobial effectiveness of pterostilbene stronger than oxacillin but pterostilbene showed bacteriostatic effect same like linezolid with MBC value exceeds the MIC.

This corresponds to [34] reported that stilbenoid compound which belongs to the phenylpropanoid family also exhibit bacteriostatic action against MRSA strains. Generally, phytochemicals or secondary plant metabolites can prevent and slow the growth of bacteria compared than kill the pathogens [35]. In this study, oxacillin and vancomycin exhibits bactericidal action against ATCC 33591 with the same value of MIC and MBC and supported by [36].

*2.2 Determination of FIC value*

The combination of pterostilbene and oxacillin exhibit partial synergistic activity against MRSA strain ATCC 33591 of the FIC values is greater than 0.5 but less than 1 which is 0.56. Pterostilbene was capable of lowering the MIC of oxacillin doubled from 62.50 µg / ml to 31.25 µg / ml against ATCC 33591. Pterostilbene in combination with vancomycin exhibit additives activity (FIC 1.00) against ATCC 33591. This suggests that pterostilbene can also act at the same site of action with vancomycin. Linezolid showed indifference activity against ATCC 33591 in the FIC of 1.25. This shows that the combined action of pterostilbene-linezolid is the same as action of pterostilbene or linezolid singly. Therefore, pterostilbene could potentially targeting different cell wall of oxacillin actions but more specifically to the site of action of vancomycin. The result of the combination between pterostilbene and three kinds of antibiotics against ATCC 33591 was shown in Tables 5.

*2.3 Result of Time-Kill Assay (TKA)*

Result of TKA for ATCC 33591, showed that growth curve of pterostilbene singly about the same as growth curve for positive control, which showed an increase in the number of bacterial colonies. Pterostilbene showed no effect either bacteriostatic or bactericidal. However, the impressive results produced when pterostilbene combined with oxacillin. This is because, although pterostilbene singly not produce a decrease in the number of bacterial colonies but when combined, this combination resulting in a decrease of bacterial colonies. Results of pterostilbene-oxacillin combination for 0.5XKS produce bacteriostatic effect (mean inhibition <3 $\log_{10}$ CFU / ml) at 4th hour with reduction of bacterial colonies of 2.29 $\log_{10}$ CFU / ml. While for concentration of 1.0XKS exhibit bactericidal effect at 16th hour with reduction of 2.13 $\log_{10}$ CFU / ml. For 2XKS, combination treatment showed bactericidal effect at 11th hour with decrease in the bacterial colonies of 1.58 $\log_{10}$ CFU / ml.

In addition, through the observation of the relationship between the rate of growth of

bacterial colonies with increasing concentrations of the treatment given showed that combination treatment of pterostilbene and oxacillin was concentration-dependent action.

Pterostilbene and oxacillin combination also produces additive interaction for all three concentrations against MRSA ATCC 33591. For oxacillin, growth curve graph showed bactericidal activity with decreasing of more than 3 $\log_{10}$ CFU / ml at the 8.5th, 16th and 8th hour at 0.5X, 1.0X and 2.0XMIC. Figure 1 (a, b, c) shows the growth curve graph for strain ATCC 33591.

Dilution of checkerboard and time-kill assay used in this study was to evaluate the antimicrobial effect of pterostilbene in combination with antibiotics. This is because, this technique provides detailed information about the type of interactions and bactericidal activity [37]. Checkerboard assay was used to determine the inhibitory effect of the combination while time-kill study was used to evaluate the bactericidal activities which rely either on time or concentration [37]. Furthermore, time-kill study gives an overview of the dynamics of antimicrobial action and interaction from time to time compared to checkerboard assay that used only once [38]. Individually, pterostilbene and linezolid displayed bacteriostatic action but pterostilbene in combination with linezolid produced indifference effect. This is consistent with studies from [32] reported that when eugenol combined with bacteriostatic antibiotic minocycline, indifference interaction effects are obtained. According to [39], indifference effect was produced when the combination action is the same as action by itself for a single component.

Pterostilbene exhibit partial synergistic effects when combined with bactericidal agents such as oxacillin against MRSA despite having same potential of anti-MRSA. Synergistic interactions showed that mechanism of action may be different [40]. This study also indicated that the interaction between pterostilbene and oxacillin can enhance partial activity of oxacillin by reducing MIC of oxacillin two-fold. In other words, an important finding in this study is pterostilbene increasing anti-MRSA activity of oxacillin. This is supported by [41] that pterostilbene act on the part of different targets of oxacillin action which is at the cell wall of bacteria and at the sites that were not involved with linezolid action on protein levels. It is also supported by the FIC study shows that pterostilbene with vancomycin against MRSA produce additive effects. Previous studies by [42] reported that combination of ellagic acid and gallic acid with β-lactam antibiotics produces additive effect. The combination of pterostilbene-vancomycin which produces an additive effect disputed by studies [43] states that the synergies was produced by the combination of ε-viniferin and vancomycin. It is also likely due to the ε-viniferin and pterostilbene has a different chemical structure. ε-viniferin do not have two groups metoxyl on benzene ring which may lead to the same site of action of pterostilbene and vancomycin.

However, there showed contradiction in the value of this two techniques, namely checkerboard technique and time-kill study. This is because, result from checkerboard technique showed partial synergistic interaction against MRSA ATCC 33591 for combination pterostilbene-oxacillin but for time-kill study found that the additive interaction was produced against MRSA ATCC 33591. It is similar to a study by [44] that showed additives activity was produced by acetone extract of *Garcinia kola* seed and methanol extracts of *Helichrysum pedunculatum* in combination with antibiotics against *Staphylococcus aureus* as a result of time-kill study [45]. Active component of the essential oil of *Thymus vulgaris* also produce additive antimicrobial activity when time-kill method was used to validate synergistic interaction that produced by FIC test [46]. The contradiction in this two technique also

supported by [47] that concluded most of the studies show contradict results for both of these techniques.

*2.4 Results of Post-Antibiotic Effect (PAE)*

The time value of PAE for the combination treatment was 2.6 hours (156 minutes) and 2.02 hours (121.2 minutes) for the treatment of pterostilbene in single and followed with the time value of PAE oxacillin singly, 0.53 hours (31.8 minutes) against MRSA ATCC 33591 (Table 6). Figure 2 shows a graph for growth rate of post-antibiotic effect (PAE) against strain ATCC 33591 of pterostilbene in combination with oxacillin, oxacillin singly, pterostilbene singly at concentrations 10XMIC for treatment singly and 10XKS for combination treatment for 24 hours.

In this study suggests that pterostilbene and oxacillin combination generates a longer time of PAE for the MRSA strain ATCC 33591. The results shown by the combination therapy against MRSA ATCC 33591 was also supported by [48,49] which demonstrated the effectiveness of antimicrobial agents can be enhanced by combining crude plant extracts with antibiotics against many pathogens, including *S. aureus*. Results from previous studies also shown that combination treatment of extracts from gall *Q. infectoria* with vancomycin exhibit longer PAE time compared to single agent against MRSA [50]. In addition, there are reports stating that aminoglycoside antibiotics produce longer PAE time on Gram-positive and Gram-negative bacteria [51]. However, this is in contrast to a study was carried out because longer PAE time was shown by combination treatment against MRSA ATCC 33591 compared to oxacillin singly.

The study by [52] reported that the beta-lactam antimicrobial produce short PAE time same as a result of this study that demonstrate short time of PAE for oxacillin singly against ATCC 33591. In addition, longer PAE time by an antimicrobial agent can be beneficial in terms of faster in killing the bacteria and likely can produce longer regimen therapeutic [53]. Studies by [54] showed that different strains exhibit different PAE time for a single treatment. PAE time for all tested strains of *S. aureus* produce a different time and length of PAE time except *S. aureus* PAE 352-3028 that showed short PAE time.

In addition, interesting discoveries from the PAE time was combination treatment in this study resulted in a longer time compared to PAE of oxacillin singly against the ATCC 33591 thus increase its potential against MRSA resistance through similar mechanism of action to oxacillin. This means that pterostilbene is synergistic to the effects of persistent antimicrobial of oxacillin against MRSA ATCC 33591. In addition, the advantage of the combination treatment was the use of a smaller dose than the dose used singly. Thus, it can reduce the toxic effect of certain medications or antibiotics [55,56,57]. TKA technique simply showed bacteriostatic effect for combination treatment and the site of action as a result of additive effect from pterostilbene and oxacillin combination.

*2.5 Observation on Morphology and Ultrastructure*

Observations of pterostilbene treatment singly using SEM against ATCC 33591 indicates the presence of protuberance or swollen on the surface of cells such as bacteria, rough surface of the cell, the cell shrinks and looks a little deformed shape of the cell (Figure 3a) compared to cells bacteria without treatment that looks smooth and rounded (Figure 5a). Observations by TEM (Figure 3b) shows the accumulation of black granules (white arrows), which are mostly located on the edge of the cell wall caused by accumulation of nucleotides, abnormal proteins or membranes that have been denatured. In addition, the cell wall also seems thicker, loss of membrane integrity and there is damage to the membrane. Cell shape also looks irregular, deformed and elongated.

The presences of *ghost* cells or cell lysates that have been observed are caused by loss of cytoplasm contents, including genetic material. In addition, the observed DNA is still clearly visible in the cell.

SEM observation for the treatment of oxacillin singly against ATCC 33591 exhibited morphological changes at the cellular level such as the presence of protuberance, swollen and rough on the bacterial cell surface (Figure 4a) compared to untreated bacteria cells that seem to exhibit a smooth cell surface (Figure 5a). In addition, bacterial cells has been shrinking and fragmented and lost its original circular structure. Observations by TEM (Figure 4b) showed that there were accumulation of black granules (white arrows), which are mostly located on the edge of the cell walls caused by nucleotide, abnormal proteins or membranes that have been denatured. Cell shape also changed, look like rods compared to the control cells (Figure 5b). In addition, the cell wall also looks thicker, loss of membrane integrity and there is damage to the membrane. *Ghost* cells or bacterial cell lyses, which was characterized by loss of cytoplasm contents including genetic material can also be observed. In addition, from observation, DNA was still clearly visible in the bacterial cell.

Evaluation of cell morphology and ultrastructure provide an overview of site of actions for each treatment which are pterostilbene, oxacillin, vancomycin, linezolid, gentamycin and ciprofloxacin singly. The bacteria were exposed to five different types of antibiotics. Antibiotics are chosen based on how their site of actions. Antibacterial agents inhibit bacterial growth through a variety of complex mechanisms, including inhibition of cell wall synthesis, cell membrane disruption, inhibition of the synthesis of nucleic acid and protein synthesis and inhibition of nucleic acid metabolism [58]. The purpose of the use of various antibiotics was to determine the site of action of pterostilbene same as the site of action of these antibiotics. Therefore, the use of various antibiotics targeting various sites on bacteria cells was used. Vancomycin and oxacillin were an antibiotic that targets cell membrane, gentamycin and ciprofloxacin are also agents that target RNA and DNA, whereas linezolid is an antibiotic that inhibits protein synthesis of the cell bacterial [21,22,23,59]. Observation of morphology and ultrastructural of the this untreated MRSA strain exhibit a spherical shape, the cell surface is smooth and like clusters of grapes. However, exposure to a variety of antibiotics and pterostilbene has changed the shape of the cell significantly compared to the untreated control cells.

The most significant morphological changes were observed in treated MRSA ATCC 33591 with pterostilbene which is the formation of protuberance and swollen on the cell surface that makes the bacterial cell looks rough. Similar results were also reported from previous studies that used extract from grape seeds. It produced effect in disrupting and damaging the cell wall that observed using transmission and scanning electron microscope [60]. Studies by [61] confirmed that the presence of protuberance because of the cell resistance to cell lysis. Increase in protuberance most likely because of bacteria mechanism to prevent damage to the plasmalemmal. Plasmalemmal damage was either one of cycle that is commonly occurred in most cells that lead to premature and necrotic death. From the observation using electron microscope showed that pterostilbene may affect the cell wall or cell membrane. Similar results were also reported by previous studies that show that the morphology of the cell wall uneven after treated of *S. aureus* with vancomycin [62].

TEM analysis with pterostilbene resulted in the presence of intracytoplasmic black granules. This can be attributed to the results of previous studies that demonstrate the presence of intracytoplasmic black

granules on treated MRSA strain with thioridazine after cultured for 18 hours [63].

This phenomenon is also likely that the presence of this structure was to prevent cells from lyses or defence mechanism of the bacterial cell. Additionally, small molecule of natural product has potential to give impact on structure of the bacterial cell. For example, natural products such as flavonoids and polyphenolic phytochemicals having the ability to inhibit the growth of bacteria by disrupting the cell membrane stability [64].

Results from observations of SEM and TEM show that changes in morphology and ultrastructure of pterostilbene is approximately the same as the changes by oxacillin. Therefore, we can conclude that the actions of pterostilbene same as action of oxacillin singly rather than other antibiotics. It is also consistent with the results of TKA and PAE which confirm additive action of pterostilbene-oxacillin combination with oxacillin singly against MRSA ATCC 33591. Next, it showed that pterostilbene act as oxacillin, which inhibits the synthesis of bacterial cell walls. This is a new discovery in identifying the site of action of pterostilbene against MRSA ATCC 33591.

## 3. Experimental Section

### *3.1 Stock Plant Compounds*

Compounds used in this study is pterostilbene that purchased commercially from EMD Biosciences / Calbiochem (USA).

### *3.2 Bacteria Study*

Bacteria used were methicillin-resistant *Staphylococcus aureus* (MRSA) which is ATCC 33591.

### *3.3 Preparation of Antimicrobial Agents*

Phytochemicals used in this study is pterostilbene that purchased commercially from EMD Biosciences / Calbiochem (USA). Stock solutions of antibiotics and phytochemicals prepared in accordance with the manufacturers recommendations. Stock phytochemicals and antibiotics were dissolved in its solvent and shaken using autovortex until the solution is dissolve completely.

### *3.4 Preparation of Bacteria Inoculum*

Bacteria inoculum preparation process begins with culturing bacteria stock obtained from the collection of Novel Antibiotic Laboratory Faculty of Health Sciences, UKM on Muller-Hinton agar (MHA) plates and incubated overnight at 37 $^0$C to obtain isolated colony. Then, three to five colonies of bacteria that grow on the agar plate were transferred into Muller-Hinton broth (MHB) using a sterile wire loop before being incubated for 24 hours at 37°C. After incubation, the turbidity of the bacterial inoculum was adjusted between 0.08 to 0.10 that equivalent to the concentration of $10^8$ CFU / ml using a spectrophotometer at a wavelength of 625nm. To achieve the desired absorption, MHB will be added to dilute the bacterial suspension. Then, the inoculation was dilute by dilution of 1: 100 to obtain inoculation size $10^6$ CFU/ml.

### *3.5 Determination of Minimum Inhibitory Concentration (MIC)*

MIC was determined using sterile 96-well microtiter plate with microbroth serial dilution method. MIC is the lowest concentration of the extract to inhibit the growth of bacteria after incubation overnight at a temperature of 37 $^0$C [65]. To facilitate observation of the growth of bacteria in each well, 2, 3, 5 - triphenyltetrazolium chloride (TTC) (2mg / ml) is dripped into each well. Bacterial growth is indicated when there is a colour change in the wells while clear or no changes in colour indicate inhibition of bacterial growth for the antimicrobial agent. This test is carried out in triplicate.

### *3.6 Minium Bactericidal Concentration Determination Test (MBC)*

MBC value was determined by culturing each clear well on MHA plates.

MBC value is determined based on the observation of the plates for the lowest concentration which shows no bacterial growth on the agar plate. This test is carried out in triplicate. The MBC is defined as the lowest concentration of an antimicrobial agent in killing 99% of microorganisms in which no bacterial growth was shown in the MHA after incubation [66].

*3.7 Determination of Fractional Inhibitory Concentration (FIC)*

Combination effect of pterostilbene and three types of antibiotics were assessed using a checkerboard method to obtain fractional inhibitory concentration index value (FIC) is either synergistic, additive, indifference and antagonistic. Both pterostilbene and selected antibiotics were prepared in five different concentrations of 1XMIC, 1 / 2XMIC, 1 / 4XMIC, 1 / 8XMIC, 1 / 16XMIC. Then, along the x-axis in 96-well microtiter plates, added 5 µl solution of pterostilbene into each well from 1 / 16XMIC, 1 / 8XMIC, 1 / 4XMIC, 1 / 2XMIC and 1XMIC. For the y-axis, 5 µl solution of antibiotics was added to each well in the same order as pterostilbene [34]. However, 40 µl of MHB were prepared in each well before pterostilbene and antibiotic solution is inserted into each well. Then, 50 µl of bacterial inoculum was added to each well and the final volume of each well was 100 µl. Positive control contain MHB and bacteria inoculum while negative control consist of MHB and distilled water or antimicrobial agents. This test is carried out in triplicate.

After that, the plates were incubated at 37 ° C for 24 hours. Turbidity or the presence of pellets in the bottom of the well indicates the presence of bacteria tested. In addition, the TTC was added to each well and incubated for 20 minutes up to two hours in a dark place. A positive result is the presence of bacterial growth that produces a colour change to pink while for negative results produced no colour change. FIC index was calculated according to the equation as follows: FIC Index = FIC A + FIC B = (MIC of drug A in combination / MIC of drug A only) + (MIC of drug B in combination / MIC of drug B only) [67]. Synergy is defined as an FIC index $\leq 0.5$, partial synergy is FIC value> 0.5 <1, additive such as FIC = 1, indifference is the FIC> 1 $\leq$ 4 and antagonistic as FIC index of more than 4.0 [68].

*3.8 Time-Kill Assay (TKA)*

Time-kill was assessed using microbroth dilution technique by [69] which use for confirmatory test of synergistic effect between pterostilbene and antibiotics. 40 µl MHB was inserted in the well of microtiter plate and then 10 µl combination agents (in ratio 1:1) with a concentration of synergistic respectively were added. Next, 50 µl of bacterial inoculum was added and make the final volume of each well is about 100 µl. Growth control only contains 50 µl of bacteria inoculum and 50 µl of MHB. This test is carried out in triplicate. Then, 96-well microtiter plate was incubated at 37 ° C and counting of bacterial colonies was performed at 0, 4, 8 and 24 hours.

At each of these times, 10 µl of samples are taken and two-fold dilution was conducted using normal saline (0.9% NaCl). After that, spread evenly over MHA using the L-shaped rod or wire loop and incubated for 24 hours at 37 $^{0}$C. Bacteria colony count was done to determine CFU / ml based on each plate consisting of 10-500 colonies. Certain dilution factors used to determine the range of the number of colonies that can be counted on petri plates. Curve of time-kill was plotted which y axis represents $\log_{10}$ CFU / ml and the x axis represents time (hour). Time-kill assay was performed using three different concentrations of 0.5X, 1.0X, 2.0X MIC and synergistic concentration (KS).

Bactericidal effect was defined as a decrease of $\geq$ 3 $\log_{10}$ CFU / ml in colony count compared to original inoculum after 24hours of incubation [70]. Synergy was

defined as a decrease of ≥ 2 $\log_{10}$ CFU / ml in colony count between the combination agents with the most active single agent after 24 hours of incubation [37]. Additive effect was defined as a reduction of <2 $\log_{10}$ CFU / ml in colony count after 24 hours of incubation for the combination agents with the most active single agent [71]. Antagonistic was defined as an increase in the number of colonies of ≥ 2 log10 CFU / mL between combination treatments with single agent that is most active after incubated for 24 hours [72].

*3.9 Determination of Post-Antibiotic Effect (PAE)*

PAE for MRSA strain ATCC 33591 was determined using colony count method that introduced by [56,73]. Treatment group is provided using pterostilbene or antibiotics singly and combination of pterostilbene-antibiotic at concentration of 10XMIC. Growth control was prepared with MHB and bacteria suspension that already dilute to $10^6$ bakteria/ml. PAE value can be generated through a comparison between the growth of bacteria with treatment and without treatment. This test was carried out by added 40 µl MHB in appendorf tube. Then, 10 µl stock solution of pterostilbene-antibiotic combination (ratio 1: 1) is inserted into appendorf tube and followed by 50 µl of bacterial inoculum. Then, this makes the final volume in the appendorf tubes 100 µl with a concentration of 10XMIC mg / ml. The same steps were used for pterostilbene and antibiotic singly.

At the same time, growth control were prepared using 50 µl MHB and 50 µl of bacteria inoculum in appendorf tube. After that, all appendorf tubes were immersed in the water bath at 37 °C for one hour. After incubation for one hour, dilution of 1: 1000 was used to remove the effect of pterostilbene or antibiotics. Similar measures were also taken to control growth. Then, 2 µl of diluted sample was spread on MHA at 0, 2, 4, 6, 8, 10 and 24 hours to allow the calculation of the number of colonies that appear after incubation for 24 hours at 37 ° C. This test is carried out in triplicate. Graf $\log_{10}$ CFU / ml were plotted against time where the PAE time can be obtained from the graph. Calculation of PAE was defined as PAE = T-C where T is the time required for bacteria of the treatment group to increase by 1 $\log_{10}$ CFU / ml after dilution. C is the time required for the control group increased by 1 log 10 CFU / ml after dilution was carried out at 1: 1000 [73]. PAE results were presented in the form of mean ± SD.

*3.10 Analysis of Electron Microscope*

This analysis was conducted to identify the site of action of pterostilbene treatment with the site of action for five types of antibiotics on changes in morphology and ultrastructural for ATCC 33591. Bacteria are exposed to five different types of antibiotics. Antibiotics are chosen based on their site of actions. Five types of antibiotics used were linezolid, gentamycin, oxacillin, ciprofloxacin and vancomycin. The purpose of the use of various antibiotics was to determine which antibiotics have the same site of action with pterostilbene. Samples for electron microscope analysis were determined using disc diffusion method [59]. Bacterial around rim of inhibition zone for each disc that will be used for electron microscope analysis.

*3.10.1 Scanning Electron Microscope (SEM)*

Bacterial cells were treated with 10% DMSO and used as a control, while the bacterial cells that treated with antibiotics used as a positive control. Bacterial cells are collected from the centrifuge and washed with distilled water. Then, binding process is done using 2% glutaraldehyde in 0.1 M phosphate buffer solution (PBS) and pH 7.4 for 15 minutes. Next, the cells are washed with distilled water for three times. After that, the binding process is carried out using 1% osmium tetroxide in distilled water for five minutes at room temperature. Samples were dehydrated using a graded ethanol series (70,

90% and 100% ethanol) for five minutes for each one. Then, coated with a thickness of 42nm gold and analyzed using a Philips XL30 ESEM (FEI Company, Oregon, USA) at 28-30 kV.

*3.10.2 Transmission Electron Microscope (TEM)*

Bacterial cells are also provided for the TEM to perform binding process using 2% glutaraldehyde in 0.1M PBS and washed with distilled water [59]. Then, staining was performed with 2% uranyl acetate for five minutes. Thereafter, bacterial cells exposed to 1% osmium tetraoxide for five minutes and the dehydration process is carried out using a series of acetone (70, 90% and 100% acetone) for five minutes for each one. Polymerization was carried out using pure epoxy resin and embedding it in the oven at 90 ° C for two hours after the bacterial cell added into a mixture of acetone and epoxy resin (1: 1) for 5 minutes. Blocks that have been trimmed will be cut into 90 nm ultrathin sections and coloured with Reynold's staining for a minute. Each specimen would be analyzed using Tecnai G2 TEM at a voltage of 100 kV.

## 4. Conclusion

Results of a study conducted by microdilution checkerboard method found that pterostilbene in combination with oxacillin produce a partial synergistic effect against tested MRSA strains. However, follow-up pharmacodynamic study showed that combination treatment produced an additive interaction and bacterisidal effect through time-kill assay against tested MRSA strains. This study also demonstrated that pterostilbene was capable in prolonging PAE time of oxacillin on both MRSA strains. This shows that test of PAE support that pterostilbene in combination with oxacillin produce a synergistic effect. The use of combination treatment can reduce the risk of toxicity, side effects from the use of antibiotics and the use of a smaller dose than the dose used singly. Moreover, the observations in the cell morphology and ultrastructural using SEM and TEM exhibit same site of action of pterostilbene with oxacillin supported by TKA test results that showed an additive effect for the combination treatment with oxacillin.

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**Conflicts of Interests**

The authors declare no conflict of interest.

**References**

[1] Bauman, R.W. Pathogenic Gram-Positive Cocci and Bacilli. *Microbiology with Diseases by Taxonomy*, 2nd Ed; Pearson Custom Publishing **2007**, 532.

[2] Pantosti, A.; Venditti, M. What is MRSA? *European Respiratory Journal* **2009**, 34, 1190–1196.

[3] Adwan, G.; Mhanna, M. Synergistic Effects of Plants Extracts and Antibiotics On *Staphylococcus aureus* Strains Isolated From Clinical Spesimen. *Middle-East Journal of Scientific Research* **2008**, 3, 134-139.

[4] Stavri, M.; Piddock, L.J.V.; Gibbons, S. Bacterial Efflux Pumps Inhibitors From Natural Sources. *Journal of Antimicrobial Chemotherapy* **2007**, 59, 1247-1260.

[5] Sucilathangam, G.; Gomatheswari, S.N.; Velvizhi, G.; Vincent, C.P.; Palaniappan, N. Detection of Antibacterial Activity of Medicinal Plant *Quercus infectoria* against MRSA Isolates in Clinical Samples. *Journal of Pharmaceutical and Biomedical Sciences* (JPBMS) **2012**, 14, 1-4.

[6] Hemaiswarya, S.; Kruthiventi, A.K.; Doble, M. Synergism Between Natural Products and Antibiotics against Infectious Diseases. *Phytomedicine* **2008**, 15, 639–652.

[7] Cowan, M.M. Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews* **1999**, 12, 564-582.

[8] Stefanović, O.; Radojević, I.; Vasić, S.; Čomić, L. Antibacterial Activity of Naturally Occurring Compounds from Selected Plants. In *Antimicrobial Agents*, Bobbarala, V.; InTech, **2012**.

[9] Ajayi, I.; Ajibade, O.; Oderinde, R. Preliminary Phytochemical Analysis of Some Plant Seeds. *Research Journal of Chemical Sciences* **2011**, 1, 58-62.

[10] Lahlou, M. Screening of Natural Products For Drug Discovery. *Expert Opinion on Drug Discovery* **2007**, 2, 697-705.

[11] Patwardhan, B.; Vaidya, A. D.; Chorghade, M. Ayurveda and Natural Products Drug Discovery. *Current Science-Bangalore* **2004**, 86, 789-799.

[12] Lahlou, M. The Success of Natural Products in Drug Discovery. *Pharmacology & Pharmacy* **2013**, 4: 17-31.

[13] Sasidharan, S.; Chen, Y.; Saravanan, D.; Sundram, K.; Latha, L.Y. Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts. *African Journal of Traditional, Complementary and Alternative Medicines* **2011**, 8, 1-10.

[14] Pan, Z.; Agarwal, A.K.; Xu, T.; Feng, Q.; Baerson, S.R.; Duke, S.O.; Rimando, A.M. Identification of Molecular Pathways Affected By Pterostilbene, a Natural Dimethylether Analog of Resveratrol. *BMC Medical Genomics* **2008**, 1, 1-13.

[15] Chong, J.; Poutaraud, A.; Hugueny, P. Metabolism and Roles of Stilbene in Plants. *Plant Science* **2009**, 177, 143-155.

[16] Seshadri, T. R. Polyphenols of *Pterocarpus* and *Dalbergia* woods. *Phytochemistry* **1972**, 11, 881-898.

[17] Maurya, R.; Ray, A.B.; Duah, F.K.; Slatkin, D.J.; Schiff, P.L. Constituents of *Pterocarpus marsupium*. *Journal of Natural Products* **1984**, 47, 179-181.

[18] Langcake, P.; Cornford, C.A.; Pryce, R.J. Identification of Pterostilbene as a Phytoalexin from *Vitis vinifera* Leaves. *Phytochemistry* **1979**, 18, 1025-1027.

[19] Pezet, R.; Pont, V. Identification of Pterostilbene in Grape Berries of *Vitis vinifera*. *Plant Physiology and Biochemistry (Paris)* **1988**, 26, 603-607.

[20] Rimando, A.M.; Kalt, W.; Magee, J.B.; Dewey, J.; Ballington J.R. Resveratrol, Pterostilbene and Piceatannol in *Vaccinium* berries. *Journal of Agricultural and Food Chemistry* **2004**, 50, 3453-3457.

[21] Domaracki, B.E.; Evans, A.M.; Venezia, R.A. Vancomycin And Oxacillin Synergy For Methicillin-Resistant *Staphylococci*. *Antimicrobial Agents and Chemotherapy* **2000**, 44, 1394-1396.

[22] Long, K.S.; Vester, B. Resistance to Linezolid Caused by Modifications at Its Binding Site on the Ribosome. *Antimicrobial Agent and Chemotheraphy* **2012**, 56, 603-612.

[23] Sieradzki, K.; Tomasz, A. Inhibition of Cell Wall Turnover and Autolysis By Vancomycin in a Highly Vancomycin-Resistant Mutant of *Staphylococcus aureus*. *Journal of Bacteriology* **1997**, 179, 2557–2566.

[24] Naja, G.; Hrapovic, S.; Male, K.; Bouvrette, P.; Luong, J. Rapid Detection of Microorganism With Nanoparticles and Electron Microscopy. *Microscopy Research and Technique* **2008**, 71: 742–748.

[25] Osafune, T.; Ehara, T.; Ito, T. Electron Microscopic Studies on Bactericidal Effects of Electrolyzed Acidic Water on Bacteria Derived From Kendo Protective Equipment. *Environmental Health and Preventive Medicine* **2006**, 11, 206–214.

[26] Reese, S.; Guggenheim, B. A Novel TEM Contrasting Technique for Extracellular Polysaccharides in *in vitro* Biofilms. *Microscopy Research and Technique* **2007**, 70, 816–822.

[27] Yamada, S.; Sugai, M.; Komatsuzawa, H.; Matsumoto, A. Suppressed Localization of a Major Autolysin on *Staphylococcus aureus* Treated with Tetracycline. *Journal of Electron Microscopy* **2001**, 50, 359–364.

[28] Diarra, M.S.; Lacasse, P.; Deschenes, E.; Grondin, G.; Paradis-Bleau, C.; Petitclerc, D. Ultrastructural and Cytochemical Study of Cell Wall Modification By Lactoferrin, Lactoferricin and Silver G against *Staphylococcus aureus*. *Journal of Electron Microscopy* **2003**, 52, 207–215.

[29] Fox, N.E.; Demaree, R.S. Jr. Quick Bacterial Microwave Fixation For Scanning Electron Microscopy. *Microscopy Research and Technique* **1999**, 46, 338–339.

[30] Nishimura, M.; Wada, M.; Akiba, T.; Yamada, M. Scanning Electron Microscopy of Food-Poisoning Bacterium *Bacillus cereus* Using a Variable-Pressure SEM. *Journal of Electron Microscopy* **2003**, 52, 153–159.

[31] Schar-Zalammaretti, P.; Ubbink, J. The Cell Wall of Lactic Acid Bacteria: Surface Constituents and Macromolecular Conformations. *Biophysical Journal* **2003**, 85, 4076–4092.

[32] Kyaw, B.M.; Arora, S.; Lim, C.S. Bactericidal Antibiotic-Phytochemical Combinations Against Methicillin Resistant *Staphylococcus aureus*. *Brazilian Journal of Microbiology* **2012**, 43, 938-945.

[33] Monte, J.; Abreu, A.C.; Borges, A.; Simões, L.C.; Simões, M. Antimicrobial Activity of Selected Phytochemicals against *Escherichia coli* and *Staphylococcus aureus* and Their Biofilms. *Pathogens* **2014**, 3, 473-498.

[34] Basri, D.F.; Luoi, C.K.; Azmi, A.M.; Latip, J. Evaluation of the Combined Effects of Stilbenoid from *Shorea gibbosa* and Vancomycin against Methicillin-Resistant *Staphylococcus aureus* (MRSA). *Pharmaceuticals* **2012,** 5, 1032-1043.

[35] Nazzaro, F.; Fratianni, F.; De Martino, L.; Coppola, R.; De Feo, V. Effects of Essential Oils on Pathogenic Bacteria. *Pharmaceuticals* **2013**, 6, 1451-1474.

[36] Singh, S.R.; Bacon, A.E.; Young, D.C.; Couch, K.A. In vitro 24-hour Time-Kill Studies of Vancomycin and Linezolid in Combination Versus Methicillin-Resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **2009**, 53, 4495-4497.

[37] Jacqueline, C.; Caillon, J.; Le Mabecque, V. *In vitro* Activity of Linezolid Alone and in Combination with Gentamicin, Vancomycin or Rifampicin Against Methicillin-Resistant *Staphylococcus aureus* by Time-Kill Curve Methods. *Journal of Antimicrobial Chemotherapy* **2003**, 51, 857–864.

[38] Eliopoulos, G.M.; Moellering, R.C. Antimicrobial Combination. In. *Antibiotic in Laboratory Medicine*. Lorian V; 3rd Ed.; Baltimore: The Williams &Wilkins Co.; **1991**, 432-492.

[39] Qin, R.; Xiao, K.; Li B.; Jiang, W.; Peng, W.; Zheng, J.; Zhou, H. The Combination of Catechin and Epicatechin Callate From *Fructus crataegi* Potentiates β-lactam Antibiotics against Methicillin-Resistant *Staphylococcus aureus* (MRSA) *in vitro* and *in vivo*. *International Journal of Molecular Sciences* **2013**, 14, 1802-1821.

[40] Bassolé, I.H.; Juliani, H.R. Essential Oils in Combination and Their Antimicrobial Properties. *Molecules* **2012**, 17, 3989-4006.

[41] Ishak, S.F.; Ghazali, A.R.; Zin, N.M.; Basri, D.F. Pterostilbene Enhanced Anti-Methicillin Resistant Staphylococcus aureus (MRSA) Activity of Oxacillin. *American Journal of Infectious Diseases* **2016**, 12, 1-10.

[42] Jayaraman, P.; Sakharkar, M.K.; Lim, C.S.; Tang, T.H.; Sakharkar, K.R. Activity and Interactions of Antibiotic and Phytochemical Combinations against *Pseudomonas aeruginosa in vitro*. *International Journal of Biological Sciences* **2010**, 6, 556-568.

[43] Basri, D.F.; Xian, L.W.; Abdul Shukur, N.I.; Latip, J. Bacteriostatic Antimicrobial Combination: Antagonistic Interaction Between Epsilon-Viniferin and Vancomycin against Methicillin-Resistant *Staphylococcus aureus*. *BioMed Research International* **2014**, 1-8.

[44] Sibanda, T.; Olaniran, A.O.; Okoh, A.I. In vitro Antibacterial Activities of Crude Extracts of *Garcinia kola* Seeds against Wound Sepsis Associated *Staphylococcus* Strains. *Journal of Medicinal Plant Research* **2010**, 4, 710–716.

[45] Aiyegoro, O.A.; Afolayan, A.J.; Okoh, A.I. Synergistic Interaction of *Helichrysum pedunculatum* Leaf Extracts With Antibiotics Against Wound Infection Associated Bacteria. *Biological Research* **2009**, 42, 327–338.

[46] Iten, F.; Saller, R.; Abel, G.; Reichling, J. Additive Antmicrobial Effects of The Active Components of The Essential Oil of Thymus vulgaris-Chemotype Carvacrol. *Planta Medica* **2009**, 75, 1231–1236.

[47] White, R.L.; Burgess, D.S.; Manduru, M.; Bosso, J.A. Comparison of Three Different *in vitro* Methods of Detecting Synergy: Time-Kill, Checkerboard, and E Test. *Antimicrobial Agents and Chemotherapy* **1996**, 40, 1914–1918.

[48] Adwan, G.; Mhanna, M. Synergistic Effects of Plants Extracts and Antibiotics On *Staphylococcus aureus* Strains Isolated From Clinical Spesimen. *Middle-East Journal of Scientific Research* **2008**, 3, 134-139.

[49] Betoni, J.E.; Mantovani, R.P.; Barbosa, L.N.; Di Stasi, L.C.; Junior, A.F. Synergism Between Plant Extract and Antimicrobial Drugs Used on *Staphylococcus aureus* Diseases. *The Memórias do Instituto Oswaldo Cruz* **2006**, 101, 387-390.

[50] Amman, V.; Basri, D.F.; Huyop, F. Determination of The Post-Antibiotic Effect (PAE) of Combinations of Extracts From Galls of *Quercus infectoria* with Vancomycin against Methicillin-Resistant *Staphylococcus aureus* (MRSA). *African Journal of Biotechnology* **2011**, 10, 18274-18278.

[51] McDonald, P.J.; Craig, W.A.; Kunin, C.M. Persistent Effects of Antibiotics on *Staphylococcus aureus* After Exposure For Limited Period of Time. *Journal of Infectious Disease* **1977**, 135, 217-223.

[52] Joseph, T.D.P.; Charles, E.E.; John, M.A.B. Pharmacodynamic of antimicrobial therapy in surgery. *The American Journal of Surgery* **1996**, 171, 615-622.

[53] Athamna, A.; Athamna, M.; Medlej, B.; Bast, D.J.; Rubinstein, E. *In vitro* Post-Antibiotic Effect of Fluoroquinolones, Macrolides, Beta-Lactams, Tetracyclines, Vancomycin, Clindamycin, Linezolid, Chloramphenicol, Quinupristin/ Dalfopristin and Rifampicin on *Bacillus*

*anthracis*. *Journal of Antimicrobial Chemotherapy* **2004**, 53, 609-615.

[54] Löwdin, E.; Odenholt, I.; Cars, O. In Vitro Studies of Pharmacodynamic Properties of Vancomycin Against *Staphylococcus aureus* and *Staphyloccous epidermidis*. Antimicrobial Agents and Chemotherapy **1998**, 42, 2739-2744.

[55] Domínguez, M.C.; de la Rosa, M.; Borobio, M.V. Application of a Spectrophotometric Method For The Determination of Post-Antibiotic Effect and Comparison With Viable Counts in Agar. *Journal of Antimicrobial Chemotherapy* **2001**, 47, 391-398.

[56] Munckhof, W.J.; Giles, C.; Turnidge, J.D. Post-Antibiotic Growth Suppression of Linezolid against Gram-Positive Bacteria. *Journal of Antimicrobial Chemotherapy* **2001**, 47, 879-883.

[57] Rybak, M.J.; Cappeletty, D.M.; Moldovan, T.; Aeschlimann, J.R.; Kaatz, G.W. Comparative *in vitro* Activities and Post-Antibiotic Effects of Oxazolidinone Compounds Eperezolid (PNU-100592) and Linezolid (PNU-100766) Versus Vancoycin against *Staphylococcus aureus*, Cougulase-Negative *staphylococci*, *Enterococcus faecalis* and *Enterococcus faecium*. *Antimicrobial Agents and Chemotherapy* **1998**, 42, 721-724.

[58] Oyaizu, M.; Fujimoto, Y.; Ogihara, H.; Sekimoto, K.; Naruse, A.; Naruse, U. Antioxidative and Antimicrobial Activity of Extracts From Several Utility Plants. *Food Preservation Science* **2003**, 29, 33-38.

[59] Santhana Raj, L.; Hing, H.L.; Baharudin O.; Teh Hamidah, Z.; Aida Suhana, R.; Nor Asiha, C.P.; Vimala, B.; Paramsarvaran, S.; Sumarni, G.; Hanjeet, K. Mesosomes are a Definite Event in Antibiotic-Treated *Staphylococcus aureus* ATCC 25923. *Tropical Biomedicine* **2007**, 24, 105-109.

[60] Al-Habib, A.; Al-Saleh, E.; Safer, A.M.; Afzak, M. Bactericidal Effect of Grape Seed Extract on Methicillin-Resistant *Staphylococcus aureus* (MRSA). *The Journal of Toxicology Sciences* **2010**, 35, 357-364.

[61] Babiychuk, E.B.; Monastyrskaya, K.; Potez, S.; Draeger, A. Blebbing Confers Resistance against Cell Lysis. *Cell Death & Differentiation* **2011**, 18, 80-89.

[62] Sieradzki, K.; Roberts, R.B.; Haber, S.W.; Tomasz, A. The Development of Vancomycin Resistance in a Patient With Methicillin-Resistant *Staphylococcus aureus* Infection. *The New England Journal of Medicine* **1999**, 340, 517-523.

[63] Martins, M.; Bleiss, W.; Marko, A.; Ordway, D.; Viveiros, M.; Leandro, C.; Pacheco, T.; Molnar, J.; Kristiansen, J. E.; Amaral, L. Clinical Concentrations of Thioridazine Enhance The Killing of Intracellular Methicillin-Resistant *Staphylococcus aureus*: an *in vivo*, *ex vivo* and Electron Microscopy Study. *International Journal of Experimental and Clinical Pathophysiology and Drug Research* **2004**, 18, 787-794.

[64] Tsuchiya, H. Biphasic Membrane Effects of Capsaicin, an Active Component in *Capsicum* Species. *Journal of Ethnopharmacology* **2001**, 75, 295-299.

[65] CLSI. Performance Standard for Antimicrobial Susceptibility Testing Twenty-Second Information Supplement. *Clinical and Laboratory Standard Institute* **2012**, 32, 1-182.

[66] Pankey, G.; Sabath, L. Clinical Relevance of Bacteriostatic Versus Bactericidal Mechanisms of Action in the Treatment of Gram-Positive Bacterial Infections. *Clinical infectious diseases* **2004**, 38, 864-870.

[67] Pankey, G.A.; Ashcraft, D.S. *In vitro* Synergy of Ciprofloxacin and Gatifloxacin against Ciprofloxacin-Resistant *Pseudomonas aeruginosa*. *Antimicrobial Agents Chemotherapy* **2005**, 49, 2959-2964.

[68] Drago, L.; De Vecchi, E.; Nicola, L.; Gismondo, M.R. In Vitro Evaluation of Antibiotics' Combinations For Empirical Therapy of Suspected Methicillin Resistant *Staphylococcus aureus* Severe Respiratory Infections. *BMC Infectious Diseases* **2007**, 7, 111.

[69] Basri, D.F.; Khairon, R. Pharmacodynamic Interaction of *Quercus infectoria* Galls Extract in Combination with Vancomycin Against MRSA Using Microdilution Checkerboard and Time-Kill Assay. *Evidence-Based Complementary and Alternative Medicine*, **2012**, 1- 6.

[70] Limoncu, M.H.; Ermertcan, S.; Cosar, G. Investigation of The Bactericidal Effects of Vancomycin and Quinupristin/Dalfopristin on *Staphylococcus aureus* Isolates. *Turkish Journal of Medical Sciences* **2004**, 34, 115–119.

[71] Aiyegoro, O.; Adewusi, A.; Oyedemi, S.; Akinpelu, D.; Okoh, A. Interactions of Antibiotics and Methanolic Crude Extracts of *Afzelia Africana* (Smith.) against Drug Resistance Bacterial Isolates. *International Journal of Molecular Sciences* **2011**, 12, 4477–4487.

[72] Lee, J.Y.; Oh, W.S.; Ko, K.S.; Heo, S.T.; Moon, C.S.; Ki, H.K.; Kiem, S.;

# The Development of Goat Meat Production in Thailand

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## Abstract

Goat meat has recently become an important aspect in the meat markets due to its containing of low fat and cholesterol contents that may benefit to human health as compared to mutton, pork and beef. Although the goat meat consumption is less as compares to chicken, pork, and beef, but the amount of world's goat meat consumption trend to be increased. In Thailand, the number of the goat population was about 26.2% increase from the year 2011 to 2015, (427,567 vs. 539,583 heads). The largest distribution of the goat population is within the Southern region (39.1%; 271,730 heads) followed by the Central region (36.6%; 209,155 heads), Northern region (19.4%; 8,876 heads) and Northeastern region (4.8%; 19,822 heads), respectively. However, goat production in this country was primarily raised for the meat purpose (95.5%; 515,093 heads). More acceptability of goat products, especially the meat was reflected a growth of Thai consumer attitude which concern more about the nutritional quality. This was due to the hard research working, and strong knowledge transferring by many organizations such as universities, private companies, Ministry of Agriculture, and the Royal projects. Nevertheless, the demand of goat meat in the market was still slowly increased probably due to the high price of meat(350 to 380 Baht or 10.9 to 11.9 $US per kg) that limit the consumer decision compared to beef (280 to 300 Baht or 8.75 to 9.38 $US per kg), and chicken (61 to 75 Baht or 1.9 to 2.34 $US per kg of chicken breast meat). In addition, goaty odour was a negative preference that consumer concern. To cope with these two main limitations, research works which had been done in Thailand within two decades were concerned more about the improvement of production and nutritional systems to increase the meat yield, whereas breeding improvement program, slaughterhouse and slaughtering protocol, goat meat quality and consumer acceptability of goat meat were also studied. Thus, studies to develop strategies for improving production efficiency of goat with minimum cost may need to be undertaken. Moreover, in order to export goat products to the halal food markets, goat products development and consumer behaviour may also need to be considered. In conclusion, it could be emphasized that the Thai goat meat industry has more opportunity in development as indicated by the increasing trend in production and consumption.

**Keywords**: Thailand, Goat meat production, Thai goat meat industry

# Bioscience to Support the Development of Local Poultry

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## Abstract

Livestock Indonesia is rich clumps local livestock but this condition has not changed much from time to time. Visible lack of attention to the development of both conventional and especially through biotechnology, so it looks very troubling because nearly all clumps of cattle has led to stagnation and extinction without serious efforts for development. To keep pace with today's attempted use of advanced biotechnology (bioscience), which are not sufficiently accurate to be implemented in Indonesia. Since its introduction the use of biotechnology in medicine, food, crops, livestock has allowed increased productivity and new products that have been utilized for the welfare of human beings (human welfare), mainly through product genetically modified food and genetically modified organisms and transgenic plant products. Now a day the application and development of bioscience in the production of meat and other poultry products, has caused consternation and barriers to the benefit of consumers. Current global consumers want meat and other food products that are free; antibiotic-free, hormone and artificial growth hormone-free, additive-free, free-Campylobacter, Salmonella-free, E.coli-free, GMOs-free, free-range, and gluten-free. It also halal food for the Muslims. Besides, in terms of the desired cultivation; animal welfare, organic back to nature animals, it is advisable for the development of local organic poultry asa implemented midtechnology. The development of local organic poultry is allowed because it was backed by traditional back yard farming and organic food that has prevailed so far by intermediate biotechnology, stay longer so organic improvement in technology in order to meet consumer needs for organic chicken is halal.

**Keywords:** local poultry, bioscience, genetically modified organism, organic chicken

## Introduction

Indonesia is rich clumps local livestock; had clumps of cattle Rooster crowing Balenggek(Ayam Kokok Balenggek), Pitalah Ducks, Ducks Bayang and Coastal Cattle (sapi Pesisir),Tegal, and Alabio ducks, Bali cattle, and others, but the conditions are not much changed from time to time until now. Visible lack of attention to the development, both naturally and especially through biotechnology, so it looks very troubling because nearly all clumps of cattle and local poultry has led to stagnation and extinction.

## Indonesia Livestock Developments

There is a tendency that a large livestock population stagnated and even declined, despite increasing demand which was finally filled by imports. This is due to the high rate cuts and declining calving interval and the reproductive problem, let alone the number of livestock farmers decreased, so that the increase in the population is not able to increase productivity, especially to meet the needs of meat is increasing every year.

Instead of poultry increased population is growing; which range chicken (1969) increased from 61.788 million into 286,690,000 birds, 668.000 laying hens tail into 98.491 million

(1.474% in 2005), broilers had reached 864.246 million in 2005, as well as ducks rose from 7,200,000 birds into 34.273 million (an increase of 476%) for 36 years. Data in 2012 also showed an increase significantly. In 2005 the population of cattle 10.68 million head and buffaloes 2.43 million heads were reported according to the census of 2011 cows and buffaloes 13.2 million head of cattle from the 2014 target of 14.3 million heads supposed to support self-sufficiency in meat, and buffalo 2. 43 million chickens and targets 4.3 million head.

According to the issue of availability of domestic meat was insufficient so the import is always increasing, and in fact often the politically issues, because investors prefer trails imports than pursue cattle and buffalo, while for farmers / ranchers effort the cattle did not excite due to low prices cow received by farmers in the country. As an illustration of the development of the livestock population Indonesia since 1969 to 2012 are presented in Table 1.

**Biotechnology in Animal Food Product Development**

Bioscience has successfully overcome the concerns of the world community will lack food source, through biotechnology. Since its introduction the use of biotechnology in medicine, food, crops, livestock has allowed increased productivity and new products that have been utilized for the welfare of human beings, mainly through product genetically modified food and genetically modified organisms and transgenic plants but not all process involves the material which is halal.

Bioscience as advanced technology is evolving through biotechnology, but apparently accompanied the rejection by consumers due to its negative impact, primarily due to gene-modified food (GMF), which can be detrimental to health. Today the application and development of bioscience in the production of meat and other poultry produks, has caused consternation and barriers to the benefit of consumers.

Table 1. Livestock Population Indonesia 1969-2012 (thousand heads).

| Jenis Ternak | 1969 | 2000 | 2005 | 2012 |
|---|---|---|---|---|
| Ruminant: | | | | |
| Beef catle | 6.447 | 11.008 | 10.680 | 16.034 |
| Milk cow | 52 | - | - | - |
| Buffalow | 2.940 | 3.109 | 2.428 | 1.378 |
| Goat | 7.544 | 11.586 | 13.182 | 17.862 |
| Sheep | 2.908 | 6.485 | 8.307 | - |
| Non Ruminant: | | | | |
| Pig | 2.875 | 9.010 | 6.267 | - |
| Horse | 642 | 585 | 408 | - |
| Poultry: | | | | |
| Local chicken | 61.788 | 229.911 | 286.690 | 264.399 |
| Layer | 688 | 54.950 | 98.491 | 130.539 |
| Broiler | - | 502.786 | 64.246 | 1.266.903 |
| Duck | 7.200 | 27.277 | 34.275 | 46.989 |

Source: Directorate General of Livestock / Animal Husbandry Statistics (2005 and 2012).

Current global consumers want meat and other food products that are free; antibiotic-free, hormone and artificial growth hormone-free, additive-free, free-Campylobacter, Salmonella-free, E.coli-free, GMOs-free, free-range, and gluten-free. It also halal-food for the Muslims. Besides, in terms of cultivation demands needs; animal welfare, organic animals and back to nature.

For the condition of Indonesia, bioscience should be directed to the improvement of the genetic quality of local livestock to increase productivity and conservation of germplasm owned local livestock, especially chickens and ducks. There are more than 39 groves of local chickens and ducks as well. In addition, to the development of quality meat food, for the application of biotechnology developed fully only would be redundant for Indonesia, especially associated with food safety. Indonesia does not require products Genetically Modified Organisms. Still, so much attention, interest and financial support from the government has not seen any.

Technology may have already mastered but the application and benefits of intermediate biotechnology are still very limited in respect of socio-economic conditions of the farmers society.

Application of biotechnology is fitting for Indonesia today, especially for cattle, buffalo, goat is; artificial insemination, estrus synchronization, embryo transfer, IVF, embryo transfer while in the field of nutrition improvement of quality of ration with prebiotics and probiotics in addition to a decrease in cholesterol as well as non-conventional feed utilization. In the field of breeding the use of microsatellites and other technologies.

Bioscience research in the field of poultry more on modern poultry, whereas for local poultry only implemented partially and not holistic due to lack of funding and coordination between the stakeholders in the field, causing the results are not yet visible to the progress of production, reproduction and productivity of the local poultry. This technology is needed to catch up in the procurement of local day old chicks bird. Since the development of biotechnology in the field of genetic, nutritional food including poultry, medical, medicine, a drastic change from a simple technology into advanced technology, efficient and helpful, but still raises questionable about their safety, especially for food for human health in addition to halal. Especially for Indonesia technological progress is still on the laboratory level, and is not generally applicable to livestock people.

**Local Poultry Potential**

Indonesia meat contribution 35.0% by cattle, bufalo and goat. Contributions of poultry reached 65.0% of total national meat production, while eggs reached 70.1% by layer of the total eggs. Although the availability of protein turned out to be close to the people the achievements of the new animal protein intake of 5.79 g / capita / day. The consumption level is much lower than the average consumption of the ASEAN average of 17.0 g / capita / day, so it can be a challenge for future business development, economic value and increased consumption.

According to Gunawan (2010), the data local chicken is not fully trusted, but the chicken population totaled 290.8 million heads. Breeding for chicken is not well coordinated. Breeding and selection by the central government in Sembawa, South Sumatra and some BPTU managed by the local government, provinces and districts are also not fully succeeded. Government programs have been implemented since 2009 in order to develop the nursery is making breeding centers managed by groups through fund nursery assistant task in several locations. In 2009 there are 5 locations 2010 and 2011 ten locations in 20 locations. While the development of the BPTU Sembawa for research and development (R & D) in order to

create strains of chicken (Poultry Indonesia, 2010). So far the project cannot support the breeding for farmers.

Local chicken has the advantage; eggs and meat taste better, demand is high and supply is often less available, the price is stable, adaptation to the environment and high tolerance to disease, tolerant of poor quality food, and the spread evenly on the maintenance of rural layer. Not lost its economic significance in the food supply of animal protein for the lower income farmers.

Carrying capacity local poultry in rural areas is getting narrower accompanied by a decline in the population characterized by;

1. Lack of back yard for range of chickens and ducks,
2. The household food waste is reduced because the technology does not leave cooking rice crust and rice casserole,
3. The average home does not have a vault which is usually a chicken coop and ducks
4. The issue of Avian Influenza
5. Rice bran is not owned by farmers, but the rice traders
6. The use of pesticides led to the chickens and ducks are no longer free to play
7. The eggs are cheaper
8. Preferences of consumers began switching to chicken
9. Culinary preferences are also changing to import food, and leaving native culinary
10. The transfer of land use
11. The high risk of failure
12. Besides yet visible effort definitely for selection and breeding of local chicken.

Local poultry will be instrumental in the development of animal husbandry in the future, along with chicken, especially in order to meet the animal protein is more natural, organic and healthy according tendency consumer preferences. With the spread of the role of maintenance in the tropical chicken then the equalization of nutrition overlooks throughout the country easily achieved, because the chicken is eaten meat, eggs and captive breeding alone. So far development is still static and experiences a variety of obstacles. The major obstacle is the provision of baby chicks to be maintained, due to limited production and compete with egg consumption, the issue of prevention of New castle disease (ND) and the issue of Avian Influenza (AI) is a lot more going on around the chicken, the issue of local food is very limited if cultivated-semi-intensive.

Local poultry development strategy depends on the purpose of maintenance; whether for Fancies ckickens that interested persons / consumers is limited, but the selling price is better, including Pelung, Bekisar, Tertawa, Ayam Kokok Balenggek chickens, and other hobbies. Another objective for the production of eggs, poultry meat that once gus for baby chicks. It required a chicken with egg production higher, rapid growth, fertility and hatchability good through breeding with the implementation of biotechnology Seeing the potential of chicken and ducks as well as its role in the countryside so high, it is for the future can be expected with the maintenance system spring or intensive, can be used as organic chickens reared according to the principles of animal walfare to improve rural welfare (Abbas, 2012) as well as free of GMOs.

Livestock development of the people through bioscience is possible but needs proper consideration for the safety of biotech food and halal. Especially for local poultry, the application of biotechnology enterprises through genetic improvement of chicken will be able to meet the expectations going organic chicken and animal welfare.

**Organic Poultry to Increase the Productivity of Small Local Poultry Farm and Human Welfare.**

Increasing concerns about food safety, health and pollution in several countries led to the demand for organic poultry products more really. This is in response to rising consumer

preference for fresh foods, free of additives, chemicals, hormones, antibiotics, and produced in accordance with animal welfare, environmental sustainability, naturally without the use of inorganic feed, so the production management system so different from conventional technology, and must be the free-range systems, natural medicament and organic feeding.

According to the small and medium sized business egg producers in local poultry, that less productivity and efficiency than large scale its better to change the business from conventional to organic poultry, because it felt more suitable for small scale businesses. Thus they do not lose capital resources, business opportunities during this especially farmers have mastered the technology, leaving only the necessary adjustments with the use of organic feed and change the system by adding a free-range-system housing. Although organic poultry less productive than conventional chicken, but with the compensation price and management poultry under the standard animal walfare, organic and ability to pay more by consumers, organic poultry remains promising as a promising venture. Organic poultry will reduce excess pollution on the environment than conventional farming (Abbas, 2012).

The main basis of our need to direct the movement towards organic poultry production, especially with the use of local chicken because of the potential and the system is relatively maintenance with free-range (back yard farming) suitable for conversion into organic poultry systems. Local chicken potency is quite significant, ranging between 300 to 330 million head of chicken than the modern layer 70-90 million head. The potential of local chickens for future development organic poultry is positive, because:

a) has been maintained from generation to generation throughout the country
b) can provide as a animal protein feed
c) the product is received by Moslem
d) the price is still relatively cheap and available everywhere.
e) There are same biotechnology for breeding and selection.

The local chickens for organic poultry it is possible because technological innovation of the integrated farming system can be relied upon by Indonesia for domestic poultry has many advantages which:

a) has the power of adaptation to local environments is high,
b) tolerant of feed quality low,
c) is more tolerant to some diseases, particularly parasitic.

Some drawbacks;

a) the genetic composition of low productivity
b) there are no obvious levels of feed inggredient,
c) there is no adequate system of breeding (Harjosworo and Prasetyo., 2009).

The oportunity to develop a local chicken as part of the organic poultry have so far hampered by;

a) Breed and breeding problem of traditional problem. In some areas there has been breeding plan but have not been able to make ends meet.
b) The number of chickens raised by farmers is relatively limited, making it difficult to provide counseling on how to raise the expected standards of organic poultry.
c) Formation of groups/cooperatives and associations of organic poultry farmers so they can act as a mentor, innovator and agencies that promote the benefits and virtues of organic poultry.
d) Fostering feed and feeding practices of local materials which are fully organic food due to the high conversion ratio.
e) The need for standardization and quality assurance bodies of organic chickens are structured.

**Development Strategy of Local Organic Chicken**

There are a number of standards and rules established for organic poultry production is more focused on aspects of feeding (Blair, 2008);

a) no genetically modified grain or grain by-products,
b) no antibiotics, hormones or drugs,
c) no animal by-products, except milk products and fish meat
d) no grain by-products unless produced from certified organic crops
e) no chemically extracted feeds (such assolvent-extacted soybean meal)
f) no pure amino acids, either synthetic or from fermentation sources.

The main objective of organic poultry is kept chickens in the condition of naturally that the production management system with technology so different from the conventional chickens. According to Blair (2008) the main difference between organic and conventional poultry others:

a) the management of chickens must use free-range system. Sistem out door enclosure allows the chickens to roam on the yard as a source of forage,
b) the genotypes of local chicken is better, because more disease resistant and adaptive to the environment.
c) the program is free to choose the right foods with average grain mixed,
d) health and animal welfare, the principle immune chickens will form naturally, but still have stringent biosecurity especially against AI (avian influenza),
e) management should be all in all out. The source of many diseases through water, soil and wild birds. Need to design a good range.

Some feed ingredients are available as a source of organic feedstuf is a local fish by products, blood meal, rumen content and many other of beans, agriculture by-product that can be upgraded through pretreatments and probiotic fermentation.

More studies are necessary to the local potential for growth and lower production, food conversion and protein efficiency is less efficient. Developed a number of enzymes for organic poultry in order to improve the utility of nutrients, rather than growth and production. Enzymes will increase the effectiveness of nutrition in the digestive tract, and reduce food substances out with eksreta, thus helping to reduce pollution.

Enzymes are commonly used extracted of plant non toxic, non-pathogenic fungi, non-pathogenic bacteria, and should not be through genetic engineering techniques, and called the exogenous enzyme. Group of enzymes commonly used and can function as probiotics are: phytase, B-glucanase, xylanase, alpha-galactosidase, alpha amylase and protease (Blair, 2008).

Some of the natural source of probiotic research conducted at the Faculty of Animal Science of Andalas University have shown results that allow the material to enhance the effectiveness of the local natural food ingredients.

**Summary**

1. Progress of bioscience who has reached genetically modified food and organisms (GMOs) has been a cause of concern in terms of food safety and halal by Muslim consumers.
2. Development of local poultry Indonesia could take advantage of the moment to developed as organic poultry, as has been maintained in the animal welfare and to improve the human welfare especially small poultry farmers to increase human welfare.

**References**

Abbas, M. Hafil., 2012. Organic poultry to improve the productivity of small scale poultry farm and human welfare in West Sumatra. Paper The 1st Poultry International Seminar 2012 " The Role of

Poultry in Improving Human Welfare", Padang September 11-12, 2012.

Blair, R., 2008. Nutrition and Feeding of Organic Poultry. Cabb International. Cromwell Press, Trowbridge.

Gunawan, D., 2010. Masih berpeluang walau ada hambatan. Laporan Utama. Poultry Indonesia, November 2010.

Harjosworo, P. S. dan L. H. Prasetyo., 2009. Unggas dan perunggasan di Indonesia. Makalah Seminar Strategi Usaha perunggasan dalam Menghadapi Krisis Global. MIPI-FAPET, 26 Oktober 2009,Bogor.

Poultry Indonesia. 2010. Industri pembibtan unggas: Tantangan dan Harapan. Laporan Utama Poulty Indonesia, Mei 2010; 11-21.

# HALAL PROBIOTICS AS HEALTH SUPPORT ISOLATED FROM DADIH

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## Abstract

Dadih is fermented buffalo milk specific from West Sumatra which containing lactic acid bacteria, so it has potential as probiotic. The use of probiotics for public health has several advantages and has a beneficial effect on human, because it is able to improve the balance of intestinal microflora. Currently in many countries, has developed a variety of probiotic products in various forms such as capsules, tablets, powders or in the form of health food, but does not guarantee that the products qualify as halal food products. In West Sumatra, there is Dadih as traditional food with specific original white and almost resembles know, have a sour taste derived from the fermentation process in a bamboo tube at room temperature for two days 28-30$^{o}$C. Dadih can be classified as halal probiotic food product because processed from buffalo milk and is a fermentation product which contains bacteria that produce lactic acid which can inhibit the growth of microbial pathogens, neutralize the pathogen bacteria in digestive tract, absorbing cancer and tumors and stimulate the immune system.

**Keywords**: Dadih, probiotics, halal products and health products

# ORAL PRESENTATION

# ANIMAL SCIENCE

# Association Analysis of NRAMP1 Gene Related to Resistance Against *Salmonella pullorum* Infection in Kampung Chicken

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## Abstract

Natural Protein Resistance-Associated Makrofag 1 (NRAMP-1) gene plays a role in controlling disease resistance. Kampung Chicken is one of the local Indonesian chickens which have high diversity in term of productivity. Breeding programs to improve disease resistance through molecular selection is one of the efforts that have evolved to increase theproductivity of chicken. The present research was to study the association of apolymorphism of NRAMP-1 with resistance disease in Kampung chicken. The PCR-RFLP method was applied to analyze the association between the polymorphism of NRAMP1 with resistant disease against*Salmonella Pullorum*. NRAMP-1 gene was genotyped in Kampung chickens using thePCR-RFLP method. The result showed three genotypes were identified of NRAMP-1 gene in Kampung chicken, namely TT, TC, and CC. The NRAMP-1 gene was polymorphic in all native chickens. The chi-square value of the Kampung chicken showed deviate in Hardy-Weinberg *equilibrium*. The TT and TC genotype revealed higher ($P<0.05$) mortality bacterium compare to CC genotype. In conclusion, polymorphism in chicken NRAMP-1 gene could be used as acandidate gene to increase resistance to disease in Kampung chicken.

Keyword: Nramp-1 gene**,** Kampung Chicken, resistance trait, and *Salmonella pullorum*

## 1. Introduction

Indonesia is one of the genetic diversity center of local chickens in the world[1].The population of local chicken from 2012 to 2015 according to Dirjen PKH [2]very small increase in the value just of 1%. Kampung chicken is a kind of Indonesian local chicken that does not have special characteristic and spread out in various regions of Indonesia. There is two big problems which become astumblingblockin developing kampung chicken. The first problem is the difficulty to get day old chick of local chicken. This problem canbe solved by integrating breeder institutions belong to thegovernment with aresearchinstitution and with local chicken producerassociation.

Most of thenative chicken were raised extensively with marginal feed, with environmental hygiene and low implementation of biosecurity. even though kampung chicken is able to thrive despite an increase in low population. Kampung chicken is resistant to several deadly diseases such as a *pullorum*. The disease is caused by the bacterium Salmonella pullorum, *Salmonella pullorum* is the cause of *pullorum* disease attacking young ages under a month with amortality rate of 20% and 80% and adult chickens act as carriers. ([3], [4]).

Resistance immune has affected theenvironment and feeds also controlled by genes. One of the genes controlledis a *Natural Resistance-Associated Protein-1* (NRAMP-1) genes. In poultry, a homologue of *NRAMP1* gene has been mapped on chromosome 7 whichconsists ofa promoter region, 15 exons, 14introns,andflankingregions5760bp in length [5], *NRAMP1* gene restricts microbial access to essential micronutrients, such as $Fe^{2+}$, $Mn^{2+}$, $Co^{2+}$, and$Zn^{2+}$,within professional phagosomes. NRAMP1 gene belongs to a large gene family encoding divalent cation transporters that are localized to late endosomes/ lysosomes and are proposed to affect intraphagosomal microbial replication by modulating divalent cation content in this organelle. The many cellular functions that depend on metal ions as cofactors may explain the pleiotropic effects of NRAMP1 and its complex role in infectious diseases ([6],[7], ([8],([9],).

The results study NRAMP-1 gene is polymorphic in broiler chickens [10], the native chicken Malaysia [11], and native chickens China ([12],[13]). Until now, variations in NRAMP1gene and their effect on disease have not been well investigated in Indonesian native chickens. Therefore, the objective of the present study was to identify the association of NRAMP1gene polymorphisms with immune traits

## 2. Material and Methods

*2.1.The time and place of study*

The study was conducted in Juli 2015 until November 2017at the Laboratory of Animal Breeding and Genetics IPB and Laboratory of Medical Microbiology Faculty of Veterinary Science IPB.

*2.2.Blood Samples*

Blood samples were 44 population kampung chicken. DNA was extracted from blood samples at the Laboratory of Animal Breeding and Genetics, Faculty of Animal Science, Bogor Agricultural University (Indonesia).

*2.3. Identification of the polymorphism NRAMP-1gene*

The first Blood samples were 44 population kampung chicken. A blood sample was taken from the brachial vein in the wing area. Identification of the polymorphism NRAMP-1gene against kampung chicken consists of 3 phases: DNA extraction, PCR amplification and RFLP (Restriction Fragment LengthPolymorphism). Genomic DNA extraction used phenol-chloroform method[14]and the DNA was dissolved in theelution buffer. The quality of the total genomic extraction was assessed by 1% agarose gel electrophoresis. Polymerase chain reaction (PCR) was carried out using primers specific for a part of exon 11 (421 bp) of NRAMP1 gene (GenBank Accession No. AY072001): forward 5'-caatgagacggtgtctgtgg-3'; reverse 5'cccagaagaaatctccctgc-3'. Amplification was carried out with a GeneAmp® PCR 9700 System (Applied Biosystems, USA). Thermal cycling conditions consisted of predenaturation at 95$^{o}$C for 5 min, followed by 35 cycles of denaturation at 95$^{o}$C for 10 s, annealing at 60$^{o}$C for 20 s, and extension at 72$^{o}$C for 30 s; the final extension step was at 72$^{o}$C for 5 min. RFLP method was used to determine the genotype NRAMP-1 gene. PCR result of the NRAMP-1 genefragments was cut bySacIrestriction enzymes.DNA amplification products and a standard DNA ladder were separated on 1.5% agarose gels in 0.5X TBE buffer.

2.4. *Clearance Test*

Immune traitswere detected in blood samples using the *clearance test* [15]. This method was used to look at normal bacterial (*S.pullorum*) population growth compared that of populations given specific treatment. The treatment impact on bacterial growth was

measured after incubating for 24-48 hat 35±1°C. Preparation of bacteria culture begins with the rejuvenation of culture in nutrient medium at a temperature of 36±1°C for 18-24 h and a sub-culture on Brain Heart Broth medium at a temperature of 36±1°C for 18-24 h.

*2.5. Data analysis*

Data were analyzed with ANOVA using completely randomized design. NRAMP-1 gene genotype was as treatment and biological assays data were as aresponse. A statistical model was used Yij = μ + Pi + εij**,** where $Y_{ij}$ is the observation on immune traits, $\mu$ is the overall mean, $P_i$ is the effect of the single nucleotide polymorphism genotypes, andεij is the random residual effect[16].

## 3. Result and Discussion

NRAMP-1 gene in chicken located on chromosome 7. The data obtained from GenBank ( GenBank accession number: AY072007). The size of NRAMP1 gene was 5760pb. The structure of this gene was begun by a promoter region, exons (15), introns (14) and theend was the flanking region.

NRAMP-1 gene *Genotyping* on exon 11, and with 421 bp PCR product. NRAMP1 gene cutting by *Sacl*enzyme restriction. The result showed two alleles (C and T). The genotype were identified of NRAMP-1 gene in Kampung chicken, namely TT, TC and CC where restriction fragments included asingle, uncut fragment of 421 bp (TT genotype), two fragments of 258and163 bp(CC genotype), and three fragments of 421, 258, and 163 bp (TC genotype) (Figure.1).

**Genotypes and allele frequency of NRAMP1 gene inkampung chickens**

Allele and genotype frequency values of NRAMP1 genes against kampung chicken presented in Table 1.

The results showed of genotyping frequency of CC genotype dominated,could be interpreted that the gene NRAMP loci SACI-1 polymorphic trait. So for [17]which states that geneticpolymorphic in anindividual can be seen when there are two alleles for the like gene but different DNA configuration which occupied the same locus on a chromo some. According to Nei and Kumar [18], polimorpysm can be indicated by the presence of two or more alleles in a population and the allele frequency is equal to or below 0.99.

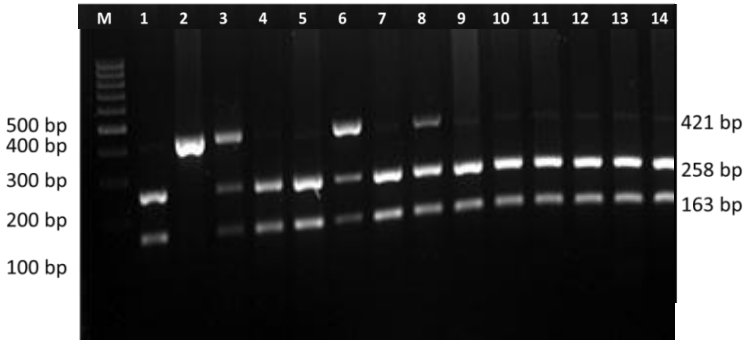


Fig. 1. PCR-RFLP amplification product of NRAMP1 gene at exon 11 that was cut by the SacI.

Table 1. Genotypes and allele frequencies of polymerase values of NRAMP1 genes against kampung chicken

| Chicken | N | Allele frequency | | genotype frequency | | |
|---|---|---|---|---|---|---|
| | | T | C | TT | TC | CC |
| Kampung | 44 | 0.24 | 0.76 | 0.14 (6) | 0.21 (9) | 0.66(29) |

**Heterozygosity and Hardy-Weinberg equilibrium Genotype Gen NRAMP1**

The results of heterozygosity values and Hardy-Weinberg equilibrium of NRAMP gene in locus *SacI* presented of Table 2. Genotype and allele frequencies The showed that the deviate in Hardy-Weinberg *equilibrium.*

Hardy-Weinberg equilibrium showed that the value of chi-squared higher than aChi-squared table at the 1% level of confidence, that the results were significantly different. In other, the ratio deviates from expectations. The higher the degree of heterozygosity in a population, the survival of the population will be higher. The degree of heterozygosity is the average percentage of loci heterozygosity of each individual or the average percentage of heterozygous individuals in the population [18].

**Resistance of Kampung Chickens**

The resistance ofkampung chickens in this study demonstrated the ability of chicken in ingesting and killing of bacteria. Ingesting killing ability has shown by clearance test with infection *S. pullorum*. The association of *NRAMP1* genegenotype with immune traits in kampung chickensare presented in Table 3.

The TT and TC genotype revealed higher ($P<0.05$) mortality bacterium compare to CC genotype. The data showed polymorphism NRAMP-1 gene in kampung chicken associated with immune traits of infection *S. Pullorum*. As a candidate disease resistance gene, NRAMP1 gene has been studied by a number of researchers throughout the world.

This study conducted by Liu et al[10], demonstrated theassociation of an SNP polymorphism in a highly conserved region of NRAMP1 with *Salmonella enteritidis* vaccine and pathogen challenge response in young chicks, indicating that either NRAMP1 or a linked gene controls these *S.enteritidis* response traits. Hu *et al* [19] report about theassociation of apolymorphism of NRAMP1 with same immune functions in chicken takes effect.

Table2. heterozygosity observations Value, expectations and Hardy-Weinberg equilibriumof NRAMP 1 gene

| Chicken | N | Ho | He | $x^2$ |
|---|---|---|---|---|
| Kampung | 44 | 0.36 | 0.21 | $8.389^n$ |

n : significantly different, $x^2$ (0.05,1) = 3.84
Ho: heterozygosity observation
He: heterozygosity expectations
$x^2$: Hardy-Weinberg equilibrium

Table 3. Association of *NRAMP1* gene genotypein kampung chickens resistant to *Salmonella pullorum*

| Genotype | Early concentration (CFU/ml) | Final concentration (CFU/ml) | Death rate of bacteria (%) |
|---|---|---|---|
| TT | $6.8 \times 10^{10}$ | $4.17 \times 10^8$ | $99,39^{ab}$ |
| TC | $6.8 \times 10^{10}$ | $1,73 \times 10^8$ | $99,74^a$ |
| CC | $6.8 \times 10^{10}$ | $7,07 \times 10^8$ | $98,96^b$ |

## Conclusion

The TT and TC genotype revealed higher ($P<0.05$) mortality bacterium compare to CC genotype. In chicken, NRAMP-1 gene could be used as acandidate gene to increase resistance to disease in Kampung chicken.

## References


[1] Sulandari, S, M.S.A. Zein, S. Priyanti, T. Sartika, M. Astuti, T. Widjastuti, E. Sujana, S. Darana, I. Setiawan, G. Garnida. *Sumber daya genetik ayam lokal Indonesia*. Bogor. vol. 45-104. 2007.

[2] Directorate General of Livestock and Animal Health. 2015. Directorate General of Livestock and Animal Health Resources Statistics2015. Directorate General of Livestock and Animal Health. Jakarta

[3] Shivaprasad, HL. *Pullorum Disease and Fowl Typhoid*. in: Disease of Poultry. SAIF, Y.M. (Ed). 11th Edition. Ames, Iowa: Iowa State Press,2003.

[4] Mcmullin, P..*A Pocket Guide to Poultry Health and Disease*. 5M Enterprises Limited. Sheffield. 2004

[5] Hu J, Bumstead N, Burke D, FA PdL, Skamene E, Gros P, Malo D. Genetic and physical mapping of the natural resistance-associated macrophage protein 1 (*NRAMP1*) in chicken. *Mamm Genome.* 6:809-815.1995.

[6] Boyer E, Bergevin I, Malo D, Gros P. Acquisition of Mn (II) in addition to Fe (II) is required for full virulence of *Salmonella enterica* serovar *Typhimurium*. *Infect. Immun.* 70: 6032-6042. 2002.

[7] Jabado N, Cuellar-Mata P, Grinstein S and Gros P. Iron chelators modulate the fusogenic properties of Salmonellacontaining phagosomes. *Proc. Natl. Acad. Sci.* 100: 6127-6132. 2003.

[8] Peracino B, Wagner C, Balest A, Balbo A. Function and mechanism of action of Dictyostelium *NRAMP1* (Slc11a1) in bacterial infection. *Traffic*. 7: 22-38. 2006.

[9] Cellier MF, Courville P and Campion C. *NRAMP1* phagocyte intracellular metal with drawal defense. *Microbes. Infect.* 9: 1662-1670.2007.

[10] Liu W, Kaiser MG, Lamont SJ.Natural resistance-associated macrophage protein 1 gene polymorphisms and response to vaccine against or challenge with Salmonella enteritidis in young chicks. *Poult. Sci.* 82: 259-266. 2003.

[11] Tohidi R, Idris I, Panandam JM and Bejo MH. Analysis of genetic variation of inducible nitric oxide synthase and natural resistance-associated macrophage protein 1 loci in Malaysian native chickens.*African Journal of Biotechnology* Vol. 10(8), pp. 1285-1289.2011.

[12] Hu JX N. Bumstead, P. Barrow, G. Sebastiani, L. Olie, K. Morgan, D. Malo. *Genome Research* 7: 693-704. 1997.

[13] He XM, Fang MX, Zhang ZT, Hu YS, Jia XZ, He DL, Liang SD, Nie QH, Zhang XQ.. Characterization of chicken natural resistance-associated macrophage protein encoding genes (*NRAMP1*and *NRAMP2*) and association with salmonellosis resistance. *Genet. Mol. Res.* 12 (1): 618-630. 2013

[14] George J. Jackson.. *Bacteriogical Analytical Manual*. Published and Distributed by AOAC International.1998.

[15] Sambrook J, Fritsch Ef, Maniatis T. Moleculer Cloning, A Laboratory Manual. 2nd ed. New York: Cold Spring Harbour Lab. Press. 1989.

[16] Mattjik AA, Sumertajaya M.. *Perancangan Percobaan dengan Aplikasi SAS dan Minitab.* Ed ke-2. Bogor : IPB Press. 2002

[17] Sofro, A.S.M. Keanekaragaman Genetik.Yogjakarta : Andi offset1994

[18] Nei, Kumar. *Moleculear Evolution and Phylogenetics.* New York: Oxford University Press.2000.

[19] Hu GS, Chang GB, Zhang Y, Hong J, Liu Y, Chen GH. Association Analysis Between Polymorphisms of Nram-1 Gene and Immune Traits in Chicken. *Journal of Animal and Veterinary Advances* 10: 1133-1136. 2011.

# Polymorphism Calpain-3 (CAPN3) Gene and Association with Carcass Traits and Meat Quality in Kampung Chicken

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## Abstract

Meat quality is one of factor associated with consumers assessment especially tenderness. One of gene that control meat tenderness is calpain-3 (CAPN3) gene.This study was aimed to identify single nucleotide polymorphisms (SNPs) CAPN3 gene in breeds of chicken. The number of chickens were used 53 kampung chicken 3 month, 46 kampung chicken 6 month,6 strain cobb, 6 F1crossbreedkampung chickens with strain cobb, 5 merawang chicken, 5 sentul chickens, 5 nunukan chickens, and 6 pelung chicken. The methods that were used the extraction of DNA from blood samples, amplification using Polymerase Chain Reaction (PCR) machine and then the SNPs were detected by DNA sequencing. Association between genotype and carcass traits and meat quality in kampung chicken was analyzed with SAS program. The results showed that CAPN3 geneintron 9 in chicken observationwere detected 2 SNPs (g.12831C>A and g.12888T>C) with 5 genotypes.SNP g.12831C>A showed in kampung chickens and F1 kampung with strain cobb. SNP g.12888T>C showed in all breeds of chicken observation. The chi-square test in all SNPs was revealed in Hardy-Weinberg equilibrium.The CAPN3 gene were notsignificantly associated with carcass traits and meat quality (P>0.05). The conclusin this study, CAPN3 gene showed SNPs atg.12831C>A and g.12888T>C and the CAPN3 gene were notsignificantly associated with carcass traits and meat quality in kampung chickens.

Keywords : CAPN3 gene, SNP, kampung chicken, carcass, meat quality

## Introduction

Improvements in growth and carcass yield are one of factor in success of chicken meat production. Selection for may have also led to changes in meat quality attributes, such as tenderness in particular [1].Meat quality traits are essential for the processing industry and end consumers [2].Meat quality were affected physical and chemical traits, age of animal, muscular and cooking methods [3]. Molecular technology approach has become a powerful method for identifying animals with particular genetic traits associated with the desired tenderness and the selection process can be done on young animals even before birth [4].

One of gene that control meat tenderness is calpain-3 (CAPN3) gene. Calpain gene is one that important to the quality of carcass and meat quality. Calpain gene is a gene whose function is to degrade protein muscle cells (myofibril) within the

muscle tissue and acts as the main enzyme in the process tenderness of meat [5]. Calpains have been reported to be involved in muscle growth and development. They are also regarded as proenzymes that are regulated by Ca2+ binding and autoproteolytic modification [6].

The CAPN3 gene consists of 24 exons located on chromosome 5 [7].The researchhave been reported that founded SNPs in the CAPN3 gene at position 11818T>A and 12814T>G on chickens in China[7] and SNPs g.15486C>T in commercial population [8]. Association genotype CAPN3 (SNP 12814T>G) were significantly with body weigth, carcass weigth, breast muscle weigth and leg muscle weigth. According to [7], needs be doing for identification of the gene SNP CAPN3 on local and kampung chicken Indonesia. In this study, we screened Single Nucleotide polymorphis (SNPs) of CAPN3 gene in native chickens in Indonesia using the method of DNA sequencing and associated with carcass traits and meat quality in kampung chicken.

## 2. Materials and Methods

The samples of chicken for polymorphisms of CAPN3 gene were used 53 chicken kampung with 3 months of age (27 cocks and 26 hens) from collection of field laboratory, faculty of animal science, bogor agricultural university , 46 chicken kampung with 6 months of age from Sukabumi, West Java, Broiler, 6 F1 kampung with broiler, 6 merawang, 5 sentul, 5 nunukan, and 6 pelung chicken. From each population were randomly sampled for collecting the blood. For carcass partial analysis were used chicken kampung with 3 months of age and chicken kampung with 6 months of age. For meat quality analysis was used chicken kampung with 6 months of age.

### *2.1 DNA extraction and Polymerase Chain Reaction (PCR)*

DNA extraction from colection of chicken blood were modified [9]. Then the extraction of DNA samplesare taken by 0.5 - 1 mL plus primer with forward 5' TCT GGT AAG GCT GAG AAA CCC 3' anf reverse 5' AAG AAA CTG CCC TGC TTC ACT C 3' by 0.35-0.4 mL, 0.3 mL of dNTPs, 1 mL of MgCl2, 1.5 mL of 10 x buffer, 0:15 Taq Polymerase and 36-46 mL of destilation water. The all mixture incubated using PCR thermocycler machine. Amplification process begins with a denaturation step at 94°C for five minutes. The second phase consists of 35 cycles, each cycle consisting of denaturation process at $94^oC$ for 10 seconds, primer annealing at temperatures range from $60^oC$ for 20 seconds and extension of DNA at a temperature of $72^oC$ for 30 seconds. The next stage is the last extension at a temperature of $72^oC$ for five minutes. The results of the DNA amplification visualized by 1.5% agarose gel.

### *2.2 DNA Sequencing*

DNA sequencing using a sequencer machine (ABI Prims 3100-Avant Genetic Analyzer) on the forward and reverse primer fragments through the 1st Base sequencing services company in Selangor, Malaysia. The results of sekeuncing analyzed with Bioedit program and MEGA6[10]

### *2.3 Statistical Analysis*

Genotype and allele frequency following model [5] was used :

$$X_{ii}=\frac{n_{ii}}{N} \qquad X_i=\frac{2n_{ii}+\Sigma n_{ij}}{2N}$$

Where Xii is the genotype frequency, Xi is the allele frequency, N is the total of sample, nii is the total of sample with genotype ii, nij is thetotal of sample with genotype ij.

Observed and expected heterozogosity following model [11] was used :

$$H_0 = \sum_{i \neq j} \frac{n_{ij}}{N} \qquad H_e = 1 - \sum_{i=1}^{q} x_i^2$$

Where Ho is the observed proportion of heterozygotes, He is the expected proportion of heterozygotes, N is the total of sample, $n_{ij}$ is total of sample with genotype ij, $X_i^2$ is the frequency of allele i, q is the total of allele.

Chi-square or Hardy-weinberg equilibrium model [12] was used :

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

Where $\chi^2$ is the Hardy-weinberg proportion, O is the observed frequency of genotype and E is the expected frequency of genotype.

Data were analyzed with GLM procedures of Statistics Analysis Sistem (SAS) Inst. Inc Cary NC, (USA).

## 3.Result and Discussion

### *3.1 PCR Amplification*

PCR amplification of the gene CAPN3 has been successfully with length products 328 base pare (bp). The CAPN3 gene fragment targets were located in exon 9 and intron 9. Visualization of PCR amplification in CAPN3 gene presented in Figure 1.

### *3.2 DNA Sequencing*

The CAPN3 polymorphisms detected by the PCR amplification were also conformed by DNA sequencing in all of observed breeds.Alignment sequences DNA of CAPN3 gene using Mega6 program presented at figure 2.

The result of alignment sequences DNA were founded 2 SNPs (g.12831C>A and g.12888T>C). SNP g.12831C>A were founded in kampung chicken with 3 months of age, kampung chicken with 6 months of ageand F1 crossbreed between kampung chicken with broiler strain cobb chicken. SNP g.12888T>C were founded in all of observed breeds.The research were reported that founded SNP in 12814T>G [7], but for this study not founded SNP 12814T>G.

### *3.3 Genotype and allele frequency of CAPN3 gene*

Polymorphysms of genes CAPN3 based on genotype and allele frequency at the SNP g.12831C>A and g.12888T>C was showed in Table 1. Position g.12831C>A were analyzed of genotype frequency CAPN3 gene, the CC genotype was the higher frequency than CA genotype frequency. The value of CC genotype frequency was showed0.83 – 1.00 and CA genotype frequency was showed0.07 – 0.10. AA genotype frequency has no value because no founded individuals with genotype of AA. Based on the position g.12831C>A, the CC genotype was the highest frequency in all chickens were observed.

The frequency of alleles at position g.12831C>A was showedthat 0.92 - 0.10 for the allele C and 0.00 - 0.04 for the allele A. The frequency of alleles at position g.12831C>A included polimorphics in kampung chicken 3 months of age, kampung chicken 6 months of age and F1 crossbreed between kampung chicken with broiler strain cobb.But, The frequency of alleles included monomorphic broiler strain cobb, sentul, merawang, nunukan and pelung chicken.

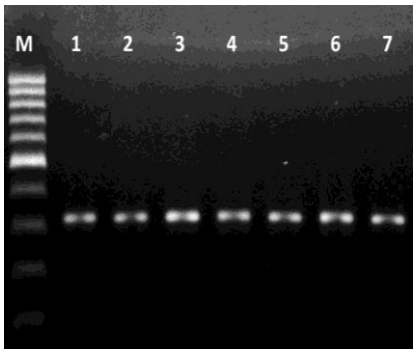


Fig 1. Results visualization amplification of CAPN3 gene in a 1.5% gel agarose. M: Marker 100 bp and 1-7

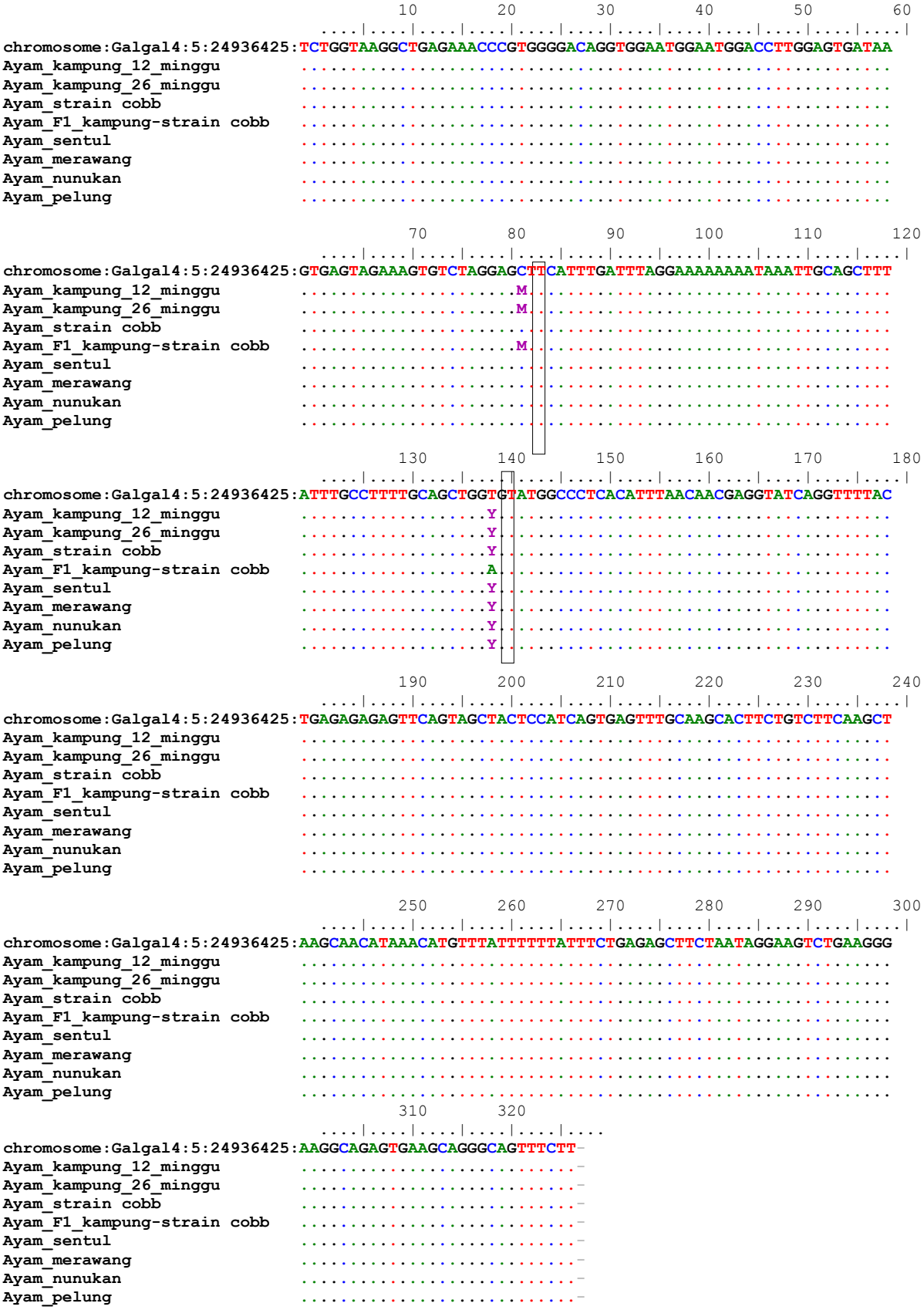


Fig. Alignment DNA sequences of CAPN3 gene in chicken

Table1. Genotype and allele frequency of CAPN3 gene in some breeds of chicken

| Breeds | N | g.12831C>A | | | | | g.12888T>C | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CC | CA | AA | C | A | TT | TC | CC | T | C |
| Kampung 3 months | 53 | 0.91 | 0.09 | - | 0.95 | 0.05 | 0.60 | 0.29 | 0.11 | 0.75 | 0.25 |
| Kampung 6 months | 46 | 0.93 | 0.07 | | 0.93 | 0.03 | 0.76 | 0.29 | 0.02 | 0.87 | 0.13 |
| Broiler Strain cobb | 6 | 1.00 | - | - | 1.00 | 0 | 0.50 | 0.33 | 0.17 | 0.67 | 0.33 |
| F1 kampung – strain cobb | 6 | 0.83 | 0.17 | - | 0.92 | 0.08 | - | 0.33 | 0.67 | 0.17 | 0.83 |
| Sentul | 5 | 1.00 | - | - | 1.00 | 0 | 0.80 | 0.20 | - | 0.9 | 0.1 |
| Merawang | 5 | 1.00 | - | - | 1.00 | 0 | 0.40 | 0.20 | 0.40 | 0.5 | 0.5 |
| Nunukan | 5 | 1.00 | - | - | 1.00 | 0 | 0.80 | 0.20 | - | 0.9 | 0.1 |
| Pelung | 6 | 1.00 | - | - | 1.00 | 0 | 0.67 | 0.33 | - | 0.83 | 0.17 |

Tabel 2. Observed heterozigosity, expected heterozigosity and Hardy–Weinberg equiblirium of CAPN3 gene in chicken

| Breeds | N | g.12831C>A | | | g.12888T>C | | |
|---|---|---|---|---|---|---|---|
| | | Ho | He | $\chi 2$ | Ho | He | $\chi 2$ |
| Kampung 3 months | 53 | 0.09 | 0.10 | Ns | 0.29 | 0.38 | ns |
| Kampung 6 months | 46 | 0.07 | 0.13 | Ns | 0.22 | 0.23 | ns |
| Broiler Strain cobb | 6 | - | - | - | 0.33 | 0.44 | ns |
| F1 Kampung – broiler strain cobb | 6 | 0.17 | 0.15 | Ns | 0.33 | 0.28 | ns |
| Sentul | 5 | - | - | - | 0.20 | 0.18 | ns |
| Merawang | 5 | - | - | - | 0.20 | 0.50 | ns |
| Nunukan | 5 | - | - | - | 0.20 | 0.18 | ns |
| Pelung | 6 | - | - | - | 0.33 | 0.28 | ns |

ns : not significant ($\chi 2$ test < $\chi 2$ table)

Table 3. Association SNP of CAPN3 gene with carcass traits in hens kampung chicken 3 months of age

| Parameter | g.12831C>A | | g.12888T>C | | |
|---|---|---|---|---|---|
| | CC (26) | CA (1) | TT (18) | TC (7) | CC (2) |
| Live weigth (g) | 663.46 ± 62.10 | 622 | 662.00 ± 44.94 | 659.86 ± 102.54 | 668.50 ± 23.33 |
| Carcass (g) | 396.69 ± 86.44 | 375 | 406.89 ± 98.62 | 382.00 ± 46.92 | 345.50 ± 10.61 |
| Breast (g) | 99.38 ± 16.69 | 113 | 102.11 ± 17.21 | 98.86 ± 15.64 | 83.50 ± 0.71 |
| Thigh (g) | 69.54 ± 8.42 | 68 | 69.33 ± 7.72 | 71.43 ± 10.05 | 64.00 ± 8.49 |
| Drum (g) | 65.85 ± 9.10 | 63 | 66.44 ± 8.26 | 66.2 ± 11.31 | 57.50 ± 2.12 |
| Wing (g) | 62.54 ± 7.88 | 65 | 62.33 ± 7.02 | 64.86 ± 10.22 | 57.50 ± 2.12 |
| Back (g) | 96.92 ± 13.74 | 102 | 97.94 ± 14.75 | 99.14 ± 5.93 | 82.50 ± 19.09 |
| Breast muscle (g) | 59.62 ± 10.13 | 54 | 60.94 ± 10.04 | 57.86 ± 10.6 | 51.00 ± 1.41 |
| Thigh muscle | 40.23 ± 5.63 | 43 | 40.11 ± 5.17 | 42.00 ± 6.88 | 36.50 ± 3.54 |
| Drum muscle (g) | 36.85 ± 5.57 | 37 | 36.78 ± 5.12 | 38.29 ± 6.80 | 32.50 ± 0.71 |

Position g.12888T>C were analyzed of genotype frequency CAPN3 gene, the TT genotype was the higher frequency than TC and CC genotype frequency in kampung chicken 3 months of age, kampung chicken 6 months of age, broiler strain cobb, sentul, merawang, nunukan andpelung chicken. But, CC genotype in F1 crossbreed between kampung chicken with broiler strain cobb The value of CC genotype was the higher frequency than TC genotype. The value of TT genotype frequency was showed 0.40 – 0.80. The value of TC genotype frequency was showed 0.20 – 0.33. The value of CC genotype frequency was showed 0.02 – 0.67. Allele frequency at position g.12888T>C was showed0.17 - 0.90 for the T allele and 0.10 - 0.83 for C allele. In all breeds of chicken included polymorphic because the allele frequency less than 0.99 [13].

### *3.4 Observed heterozigosity, expected heterosity and Hardy-Weinberg in CAPN3 gene*

Values of observed heterozygosity, expected heterozigosity and Hardy Weinberg equilibrium CAPN3 genes was showed in Table 2. Observed heterozygosity included the position g.12831C>A CAPN3 gene in kampung chicken 3 months of age and 6 months was higher than expected heterozygosity. SNP g.12831C>A in F1 crossbreed between kampung chicken with broiler strain cobb strain showedobserved heterozygosity higher than expected heterozygosity.

CAPN3 gene position g.12888T>C, observed heterozygosity was higher than expected heterozygosity in kampung chicken with 3 months of age, kampung chicken with 6 months of age, broiler strain cobb, merawang chicken. But, expected heterozygosity was higher than observed heterozygosity in F1 crossbreed kampung chicken with broiler strain cobb, sentul, nunukan and pelung chicken.A population was a high genetic diversity if it has a value of heterozygosity more than 0.50. If value of expected heterozigosity was higher than observed heterozigosity in different populations indicatedhigh random mating[14]. Chi-square test at SNP g.12831C>A was showed in hardy-weinberg equiblirium in kampung chicken with 3 months of age, kampung chicken with 6 months of age and F1 crossbreed kampung chicken with broiler strain cobb. Chi-square test at SNP g.12888T>C was showed in hardy-weinberg equiblirium in all breeds of chicken.

### *3.5 Association CAPN3 gene with carcass and meat quality in kampung chicken*

SNP genotypes were associated with an carcass traits in chicken kampung with 3 months of age and 6 month of age were summarized in Table 3, 4 and 5.

Analisys association CAPN3 gene were not significantly ($p>0.05$) with carcass traits in hens kampung chicken 3 months of age, cocks kampung chicken 3 months of age and cocks kampung chicken 6 months of age. The reason for this contradictory because be due to type of mutation in SNP g.12831C>A and g.12888T>C.Type of mutation in SNP g.12831C>A is transversions and Type of mutation in SNP g.12831C>A is transitions. The result can be caused slow growth in kampung chicken in each genotype SNP. So, the average of carcass traits were not different significantly.

### *3.6 Association of CAPN3 SNP with meat qualityin kampung chicken*

The result of association analysis by using the GLM between the CAPN3 gene polymorphism with meat quaity in kampung chicken were showed in Table 6. Result of association the all SNPs of CAPN3 gene not signicificant were detected for meat quality (pH, cooking loss, water holding capacity, tenderness and fat) in kampung chicken.

Table 4. Association SNP of CAPN3 gene with carcass traits in cocks s kampung chicken 3 months of age

| Parameter | g.12831C>A | | g.12888T>C | | |
|---|---|---|---|---|---|
| | CC (22) | CA (4) | TT (14) | TC (8) | CC (4) |
| Live weigth (g) | 766.5 ± 113.53 | 753.00 ± 145.85 | 748.93 ± 95.20 | 748.63 ± 112.63 | 850.25 ± 176.69 |
| Carcass (g) | 447.55 ± 91.19 | 447.50 ± 91.08 | 428.36 ± 78.24 | 459.13 ± 85.70 | 491.50 ± 134.84 |
| Breast (g) | 113.32± 25.19 | 114.25 ± 22.23 | 108.50 ± 23.92 | 115.88 ± 20.24 | 126.00 ± 34.06 |
| Thigh (g) | 83.86 ± 15.94 | 82.75 ± 21.85 | 80.86 ± 12.34 | 83.63± 17.85 | 93.75 ± 26.09 |
| Drum (g) | 81.14 ± 15.18 | 81.25 ± 17.75 | 76.64 ± 11.54 | 83.63 ± 14.93 | 92.00 ± 23.73 |
| Sayap (g) | 73.77 ± 11.62 | 73.75 ± 8.06 | 72.50 ± 9.05 | 74.75 ± 9.94 | 76.25 ± 20.16 |
| Back (g) | 112.55 ± 24.17 | 108.00 ± 27.12 | 107.50 ± 22.57 | 112.00 ± 24.64 | 126.75 ± 28.96 |
| Breast muscle (g) | 65.95± 16.29 | 65.25 ± 16.76 | 60.93 ± 14.22 | 68.25 ± 15.11 | 78.25 ± 19.92 |
| Thigh muscle (g) | 48.09 ± 12.92 | 48.00 ± 11.91 | 45.64 ± 11.61 | 49.63 ± 12.53 | 53.50 ± 16.90 |
| Drum muscle (g) | 44.91 ± 8.54 | 39.75 ± 5.74 | 42.79 ± 7.55 | 45.5 ± 9.20 | 46.00 ± 10.55 |

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Table 5. Association SNP of CAPN3 gene with carcass traits in hens kampung chicken 3 months of age

| Parameter | g.12831C>A | | g.12888T>C | | |
|---|---|---|---|---|---|
| | CC (43) | CA (3) | TT (36) | TC (9) | CC (1) |
| Live weigth (g) | 1626.09 ± 113.49 | 1532.67 ± 60.86 | 1642.06 ± 115.13 | 1542.00 ± 59.24 | 1525 |
| Carcass (g) | 1038.65 ± 100.78 | 972.00 ± 33.78 | 1054.67 ± 99.57 | 962.00 ± 56.52 | 952 |
| Breast (g) | 263.98 ± 26.69 | 252.67 ± 4.04 | 267.75 ± 26.92 | 246.11 ± 13.72 | 255 |
| Thigh (g) | 203.72 ± 25.95 | 185.33 ± 13.20 | 208.19 ± 25.21 | 181.44 ± 15.74 | 188 |
| Drum (g) | 190.40 ± 22.96 | 168.67 ± 3.21 | 193.64 ± 22.81 | 172.44 ± 14.47 | 170 |
| Wing (g) | 131.53 ± 11.34 | 124.00 ± 9.17 | 132.31 ± 11.57 | 127.00 ± 9.70 | 122 |
| Back (g) | 256.98 ± 38.60 | 257.33 ± 17.79 | 262.56 ± 34.50 | 236.89 ± 45.31 | 238 |
| Breast muscle (g) | 185.00 ± 25.41 | 179.67 ± 13.58 | 186.97 ± 26.33 | 177.33 ± 16.98 | 167 |
| Thigh muscle (g) | 149.67 ± 24.76 | 133.00 ± 18.52 | 153.92 ± 24.37 | 130.56 ± 14.12 | 119 |
| Drum muscle (g) | 125.67 ± 19.12 | 116.33 ± 9.29 | 128.36 ± 19.31 | 111.67 ± 9.38 | 127 |

Table 6. Association of CAPN3 gene with meat quality in kampung chicken

| Parameter | g.12831C>A | | g.12888T>C | | |
|---|---|---|---|---|---|
| | CC (43) | CA (3) | TT (35) | TC (10) | CC (1) |
| pH | 5.44 ± 0.27 | 5.48 ± 0.11 | 5.45 ± 0.22 | 5.42 ± 0.36 | 5.61 |
| Cooking Loss (%) | 48.41 ± 2.21 | 46.38 ± 1.09 | 48.10 ± 2.31 | 48.83 ± 1.82 | 45.15 |
| Water Holding Capacity (%) | 29.94 ± 2.18 | 29.01 ± 0.57 | 29.82 ± 2.33 | 30.32 ± 1.13 | 28.40 |
| Tenderness ($kgcm^{-2}$) | 2.91 ± 0.70 | 3.08 ± 0.50 | 2.93 ± 0.73 | 2.93 ± 0.60 | 2.90 |
| Fat (%) | 0.83 ± 0.60 | 0.41 ± 0.25 | 0.82 ± 0.62 | 0.78 ± 0.54 | 0.15 |

## Conclusion

The gene of CAPN3 founded 2 SNPs in intron 9 (g.12831C>A and g.12888T>C). SNP g.12831C>A is polymorphism in kampung chicken and F1 kampung chicken with strain cobb with CC and CA genotype. SNP g.12888T>C is polymophism in all breed of chickens with TT, TC and CC genotype. Gene CAPN3 no significantly associated with carcass traits and meat quality in kampung chicken.

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## References

[1] E. Dransfield and A. A. Sosnicki. Relationship between muscle growth and poultry meat quality. Poult. Sci. 78: 743-746.Agust.1999.

[2] G. B. Park, S. S. Moon, Y. D. Ko, J. K. Ha, et al. Influence of slaughter weight and sex on yield and quality grades of Hanwoo (Korean native cattle) carcasses. J. Anim. Sci. 80: 129-136.May, 2002.

[3] Soeparno. Ilmu dan Teknologi Daging. Yogyakarta: Gadjah Mada University Press.2009.

[4] J.T. Shun, M. Zhang, Y.J. Shan, W.J. Xu, K.W. Chen and H.F. Li. "Analysis of the genetic effects of CAPNl gene polymorphisms on chicken meat tenderness". Genet. Mol. Res. Vol. l4, no. l, pp. l393- l403, Feb. 20l5.

[6] D.E. Goll, V.F. Thompson, H. Li, and W. Wei. The calpain system. Physiol. Rev. Vol. 83, pp. 73l-80l.July, 2003.

[5] Koohmaraie M. Biochemical factors regulating the toughening and tenderization processes of meat. *Meat Sci*. Vol.43: 193-201, 1996.

[7] Z. R. Zhang, Y. P. Liu, Y. G. Yao, X. S. Jiang, H. R. Du and Q. Zhu. Identification and association of the single nucleotide polymorphisms in calpain3 (CAPN3) gene with carcass traits in chickens. *BMC genetics*. 10:10, March 2009.

[8] Felicio M A, C. Boschiero, J C C Balieiro, M C Ledur, J B S Ferraz, T M Filho, A S A M T Moura, L L Coutinho. Identification and association of polymorphisms in CAPN1 and CAPN3 candidate genes related to performance and meat quality traits in chickens. *Genetics and Molecular Research* 12 (1): 472-482, Feb. 2013.

[9] J.Sambrook and D. Russell. Molecular Cloning: A Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press, United State of America.2001.

[10] K.Tamura, G. Stecher, D. Peterson, A. Filipski,and S.Kumar. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Bio. Evol.*30 :2725-2729, Oct. 2013.

[11] M. Nei and S. Kumar. *Molecular Evolution And Phylogenetics*. New York: Oxford Univ Press. 2000.

[12] R. R. Noor. *Genetika Ternak*. Jakarta: Penebar Swadaya.2010.

[13] D. L.Hart and Clark AG. *Principles of Population Genetics*. 3rd ed. Sunderland: Sinauer Associate Inc. 1997.

[14] F. W. Allendorf, GLuikart. *Conservation and The Genetics of Populations*. Oxford (GB) : Blackwell Publishing. 2007.

# Physical Quality of Broiler Meat Fed Diets Containing Mealworm Protein Concentrate

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## Abstract

The research aimed to evaluate the physical quality of broiler meat fed with diets containing mealworm protein concentrate. Mealworms are beetle larvae of *Tenebriomolitor*, local insects that are potential to be used as feed material in Indonesia and expected to replace Meat and Bone Meal (MBM) that are still imported. Mealworm protein content were higher than the MBM, about 45.87% and 42.94%, respectively. The materials used for this research were 100 DOC (Day Old Chick) Lohman strain (MB 202) broiler male with average body weightof 46.72±1.32 g/ head. The broilers were reared for 35 days. The experimental design was Completely Randomized Design (CRD) with two treatments and five replications, and each replication consisted of ten broiler chickens. The treatments consisted of T0 and T1. T0 was feed containing mealworm protein concentrate0% and 5% MBM as a control and the T1 was feed containing 5% mealworm protein concentrateand 0% MBM. Data were analyzed using T-test. The variables measured were pH, Tenderness, Water Holding Capacity (WHC), and cooking loss. The result showed that feed with mealworm protein concentrates had no significant effect ($P < 0.05$) on tenderness and cooking loss. While, T1 had higher pH and WHC value than T0. It can be concluded that the utilization of mealworms protein-containing feed can replace MBM and showed better influence on the physical quality of broiler meat (pH, tenderness, cooking loss, and WHC).

Keywords: mealworm, protein, physical quality of the meat, broiler

## 1. Introduction

Mealworm or *yellow mealworm* is beetle larvae of *Tenebriomolitor* [1]. In Indonesia, it is popular as "ulathongkong" (hongkongcaterpillar). Mealworm is one of the local insects that could potentially be used as feed material in Indonesia. Reference [2] reported that mealworm contains good nutrients including protein, fat, carbohydrates and vitamins. In addition to having a high nutrient, mealwormis also easily found, can grow quickly, and the feed is not complicated. It also requires a low input in breeding.

Some studies related to mealworm have been reported. Reference [3] found that mealworm contained complete essential amino acid composition. Mealworm has been industrially produced as feed for pets and animals at the zoo, including birds, reptiles, small mammals, amphibians and fish [4]. This caterpillar has proven to be a source of protein that can be accepted by the African catfish

[5], chicks [6], laying hens [7], and pork [6]. Reference [6] used mealworm as a substitute soybean meal in feed chicks. In Europe, the mealwormhas been widely used not only as animal feed, but also human consumption [1].

Reference [9] showed that mealworm can be used as an alternative to imported feed materials in Indonesia, namely *Meat Bone Meal* (MBM) in broiler chicken feed. Substitution of MBM by mealworm is considerable since it has higher protein (45.87%) than MBM (42.94%). Mealworm in the form of extract and flour could increase its protein content. The use of mealworm intact in broiler chicken feed unaltered the performance, but no research has been done related to the quality of meat and protein extraction of mealworm [10]. The physical quality of the meat shows great effects on consumer acceptance. Physical or chemical quality of the meat were affected by the feed [11]. Therefore, this study was conducted to evaluate the use of the mealworm protein concentrates to the quality of broiler meat.

## 2. Material and Methods

The research was conducted at the cages of Animal Husbandry Faculty, Bogor Agricultural University in July-September 2016. The physical quality analysis was conducted at the Laboratory of the Department of Ruminant.

This study used 100 DOC *(Day Old Chick)* male broiler chickens with brands MB 202 *(Lohmanstrain)* that were divided into two treatments and five replications, and each replication consisted of 10 animals.

### *2.1.Feed Production*

Mealworm protein was extracted using dry and rendering methods [12]. The composition of feed ingredients in the ration was exhibited in Table 1. Feed on starter and *finisher* phase was formulated according to [13]. Details of treatment were:

R0 : Feed containing mealworm protein concentrate 0%, MBM 5% (control)

R1 : Feed containing mealworm protein concentrate5%, MBM 0%

### *2.2.Maintenance and Slaughter*

Maintenance of broilers was conducted for 35 days. The feeding and drinking method was performed *ad libitum,* 3 times a day(at 07.00 (morning), 12.00 (noon)and 16.00(afternoon). At the end of the experiment, the chickens were slaughtered after fasting for 24 hours. Chicken samples were taken at random, respectively 10% in each plot. Samples were taken on the breast to test the quality of the meat.

### *2.3.Physical Quality of Broiler Meat*

pHvalue [14]. Measurement was performed using pH meter (Hanna, USA). Cathode was calibrated, and then dipped in the sample to obtain constant pH value displayed in the pH meter. Cathode was rinsed with distilled water for next measurement.

Tenderness [11]. The meat was boiled at 80 $^0$C. The meat samples were drained and cooled for 1 hour to obtain constant weight. Samples were formed by following the directions *correr* meat fibers. Strips of flesh was measured by *Warner-Blatzer Shear Force* to determine the value of the power breakdown.

Cooking Loss [15]. Cooking loss represented the weight ratio of pre-cooked and post-cooked meat at 80 °C for 15 minutes.

WHC [15]. Hamm methods can be used to describe the WHC of meat. Samples (0.3 g) were set between filter papers and pressed with load 35 kg. After 5 minutes, the wet area surrounding the covered area was marked and measured.

Table 1. The composition of feedstuffs treatment (starter and finisher)

| Material Composition | Starter | | Finisher | |
|---|---|---|---|---|
| | R0 (%) | R1 (%) | R0 (%) | R1 (%) |
| Corn | 54.18 | 54.09 | 63.77 | 64.70 |
| Rice bran | 3.50 | 4.00 | 3.50 | 3.50 |
| Soybean meal | 30.81 | 29.78 | 20.78 | 19.69 |
| **MBM** | **5** | **0** | **5** | **0** |
| **Mealworm protein concentrate** | **0** | **5** | **0** | **5** |
| CPO | 4.04 | 3.00 | 4.59 | 3.20 |
| $CaCO_3$ | 0.85 | 1.50 | 0.66 | 1.35 |
| DCP | 0.30 | 1.35 | 0.45 | 1.33 |
| Salt | 0.45 | 0.44 | 0.45 | 0.41 |
| L-Lysine | 0.08 | 0.18 | 0.09 | 0.19 |
| DL-Methionine | 0.28 | 0.22 | 0.21 | 0.13 |
| Premix | 0.50 | 0.50 | 0.50 | 0.50 |
| Total | 100 | 100 | 100 | 100 |
| ***Nutritional Content:** | | | | |
| Dry ingredients (%) | 89.63 | 89.87 | 89.69 | 89.88 |
| Metabolizable energy (kkal/kg) | 3050 | 3050 | 3150 | 3150 |
| Crude protein (%) | 22 | 22 | 18 | 18 |
| Fat (%) | 6.94 | 6.43 | 7.79 | 6.96 |
| Crude Fiber (%) | 2.82 | 3.32 | 2.76 | 3.22 |
| Calcium (%) | 0.95 | 0.97 | 0.89 | 0.89 |
| Phosphor (%) | 0.68 | 0.66 | 0.67 | 0.61 |
| Lysine (%) | 1.30 | 1.30 | 1 | 1 |
| Methionine (%) | 0.65 | 0.57 | 0.52 | 0.43 |
| Methionine+Cystine (%) | 0.95 | 0.96 | 0.76 | 0.75 |

*The Result of Calculation

Table 2. Chicken carcass as result of mealworm protein concentrate addition

| Parameters | Feed Containing | |
|---|---|---|
| | MBM | Mealworm |
| Final Body Weight (g) | 1631 ± 62 | 1680 ± 72 |
| Carcass weight (g) | 1093 ± 53 | 1159 ± 60 |
| Carcass percentage (%) | 67 | 69 |

Table 3. Physical quality of broiler meat treated with addition of mealworm protein concentrate

| Parameters | Feed Containing | |
|---|---|---|
| | MBM | Mealworm |
| pH | 5.95 ± 0.04a | 6.19 ± 0.12b |
| Tenderness | 7:01 ± 0.48a | 6.69 ± 0.61a |
| Cooking Loss (%) | 35.66 ± 6.89a | 32.96 ± 6.08a |
| WHC (%) | 23:19 ± 2.74a | 34.02 ± 3.21b |

The different letters following the values in the same row indicate significant difference ($P < 0.05$)

Wet area was outside of the circle, the circle area was measured using planimeter to obtain $mgH_2O$.

$$mgH_2O = \frac{\text{wet area } (cm^2)}{0.0948} \times 8.0$$

*2.3.Statictical Analysis*

Completely randomized design was selected for experimental design. The data were analyzed by t-test using SPSS software.

## 3. Result and Discussion

Percentage of broiler carcass with treatment of mealworm protein concentrate is presented in Table 2.

The results showed that final body weight of chicken was not different. However, the percentage of chicken carcass with treatment of mealworm protein concentrate has a higher value. Chicken carcass as result of mealworm protein concentrate additionwere evaluated it's meat quality.

Meat quality is attribute to high brightness and not stink. In addition, pH, tenderness, cooking loss and water holding capacity (WHC) of meat contributed to meat quality in term of consumer acceptance [11]. The physical quality of broiler chicken meat is displayed in Table 3.

*3.1. Potential Hydrogen (pH)*

Table 3 indicates that the administration of feed containing mealworm protein concentrate shows significant differences ($P < 0.05$) in pH values of male broiler chicken breast meat. After cutting process, the pH value of broiler meat was 6.31, and then decreases [16]. The pH in this study was measured at three hours after cutting.Therefore, the pH value is not extremely decreased. The absence of blood after the slaughtering discontinues oxygen supply to the brain, and causes glycogen scarcity in the muscles.Consequently, some changes are observed in the muscle including temperature, pH, and process of rigormortis. The decrease in pH value was associated with high acidity due to production of lactic acid. This condition led to formation of open structure of the meat and lowered the WHC [17]

*3.2. Tenderness*

Table 3 showed that the treatments (MBM and Mealworm protein concentrate) had no significant effects ($P > 0.05$) on the meat tenderness (7.01±0.48 and 6.69±0.61, respectively). Tenderness represented the pressure needed to cut a product per unit area (kg cm-2), suggesting that the smaller value showed more tenderness of the meat [11]. The higher value of *Warner Blatzler* means more force required to break the fibers of the meat square percentimeter. This means high meat tenderness [18]. The level of meat tenderness was determined by the extent of connective tissue proteins, namely collagen, miofibril, actomyosin and elastin [16].

In the study, we tested breastmeat to produce relatively equal tenderness. The breastmuscleis considered to have low activity, thus the breast muscle is less structured. In addition, less myofibril protein meat lead to lower water holding capacity, contributing to higher meat tenderness. This is in accordance with [19] that the high activity muscles have greater myofibril fibers, and proteins in the muscle meat as liaisons have an important effect on the value of meat tenderness. The higher the protein content of meat, the meat tenderness is lower.

*3.3. Cooking Loss*

T-test showed that the treatments showed no significant effects on percentage of cooking loss ($P > 0.05$). Cooking loss is affected by the protein content of feed. The feed has similar protein content, 22% in the starter phase and 18% in the finisher phase (food prepared in iso protein), resulting in similar cooking loss. However, Table 3 indicated that feed containing mealworm

protein concentrate tend to produce lower cooking loss. Reference [11] reported that desirable meat had low cooking loss due to less nutritional disintegration. This figure relates to the binding power of water by protein meat. Low WHC of meat is attributed to high fluid loss, resulting in decreased meat weight. In this study, the cooking loss is in acceptable value.Acceptable values of cooking shrinkage of chicken meat was 15-40% [16].

*3.4. Water Holding Capacity (WHC)*

Water holding Capacity (WHC) is the ability of the meat protein to bind water. Thus, WHC, expressed as percent, represents the level of meat protein damage. The ability to bind water is an important factor, especially in the meat to be used in the food industry. Administration of mealworm protein concentrate in the feed showed significant differences ($P < 0.05$) as exhibited in Table 3. Reference [19] found that the decrease rate of pH in *postmortem* meat is a key determinant for water holding capacity. The pH value and the value of WHC was inversely correlated in this study. This is consistent with the statement [11] that the pH value of meat is directly proportional to the water holding capacity.The higher the pH value of the meat, the greater the ability of meat to bind water, vice versa. Furthermore, reference [20] suggests that changes in water holding capacity of meat due to the change ions are bound by protein meat. The decline in water holding capacity is due to the increasing number of lactic acid that accumulates as a result of damaged miofibriler proteins. Thus the ability of protein to bind water is reduced. High protein content of meat is attributed to higher water binding power. The expansion of the network of proteins or protein miofibril development (particularly myosin) was due to the weakening of hydrogen bonds or hydrophobic bonds that cause more mobilized between miofibril, resulting in higher water holding capacity [20].

## Conclusion

Feed supplementation by meal worm protein concentrates was considerable to substitute MBM, and exhibited better effects on physical properties of broiler meat.

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## References

[1] [EFSA] European Food Safety Authority. Risk profile related to production and consumption of insects as food and feed. *J.Efsa*.2015.4257.2015.

[2] De Foliart G, Dunkel FV, Gracer D. The food insect newsletter-chronicle of changing culture. *Salt Lake City: Aardvark Global Publishing.* p.ix+414.2009.

[3] Finke MD. Complete nutrient composition of selected invertebrates commonly fed to insectivores. *Zoo Biology*. 21.269–285.2002.

[4] Aguilar-Miranda ED, López MG, Escamilla-Santana C, Barba de la Rosa AP. Characteristics of maize flour tortilla supplemented with ground *Tenebriomolitor*larvae. *J Agric Food Chem*. 50 (1): 192-195.2002.

[5] Ng K, Liew FL, Ang LP, WongKW. Potential of mealworm (Tenebromolitor) as an alternative protein source in practical diets for African catfish,*Clariasgariepinus*. *Aquac. Res.* 32, 273–280.2001.

[6] Ramos-Elorduy J, Gonzalez EA, Hernandez AR danPino JM. Use of Tenebriomolitor (Coleoptere: Tenebrionidae) to recycle organic wastes and as feed for broiler chickens. *Journal of Economic Entomology*. 95. 214–220.2002.

[7] Giannone M. A natural supplement made of insect larvae.*J. Rivista di Avicoltura* 72 (38). 40–41.2003.

[8] Newton L, Sheppard C, Watson DW dan Burtle G. Dried *Hermetia illicens* larvae meal as suplement for swine. *J. Anim Sci*. 44. 395-400.2005.

[9] Purnamawati Y. *Performance of broiler chickens fed containing mealwarm instead of Meat and*

*Bone Meal in broiler chickens.[*skripsi]. Bogor (ID): Bogor Agriculturer University. 2015

[10] Novriansayah E. *Performance of broiler chickens fed starchy instead of Meat and Bone Meal in broiler chickens [thesis]*. *Bogor (ID):*.[Skripsi]. Bogor (ID): Bogor Agriculturer University.2015.

[11] Soeparno. M*eat Science and Technology*. Yogyakarta[ID]: UGM Pr. 2005.

[12] Andarwulan, N., F. Kusnandar, dan D. Herawati. Meat science.. PT Dian Rakyat, Jakarta.2011.

[13] Leeson S dan Summers JD. *Commercial Poultry Nutrion3th Edition*. Canada [Cnd]: Nottingham University Pr.2005.

[14] [AOAC] Association Official Analitycal Chemistry. *Official Method of Analysis*. 18th Ed. Maryland (US): AOAC International.2005.

[15] Smith DP, Northcutt JK, dan Steinberg EL. Meat quality and sensory attributes of a conventional and a Label Rouge-type broiler strain obtained at retail. *Poultry Sci* 91 :1489–1495 http://dx.doi.org/ 10.3382/ps.2011-01891.2012.

[16] Aberle HB, Forrest JC, Hendrick ED, Judge MD, Merkel RA. *Principle of Meat Science*. Ed ke-4.Dubuque (US): Kendal/Hunt Publishing Co.2001.

[17] Buckle, K. A., R. A. Edwards, G. H. Fleet, and F. M. WootonMeat Science. Translator;Purnomo, H. danAdiono. Cetakan Ke-1. University of Indonesia Press, Jakarta.1985.

[18] Suryati T, Astawan M, Wresdiyati T. Physical Properties of Meat Sheep were given treatment Electrical Stimulation Low Voltage and Injection of Calcium Chloride.*Media Peternakan*. Vol. 27.no. (3). p.101-106.2004.

[19] Lawrie RA. *Meat Science*. Translator: AminudinParakasi. UI-Press. Jakarta2003..

[20] Hamm R. Postmortem change in muscle affecty the quality of comminuted meat products.*Meat Sci*.1981.

# Identification Polymorphisms of Inos Gene and Association with Body ResistanceTrait in Kampong Chicken

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## Abstract

INOS gene is one of the genes that play important factor in immunity related to resistence disease through non specific immune response in chicken. The aim of this study was to identify the polymorphism of iNOS gene in kampong chicken and it is association with resistance to the bacteria *Salmonella pullorum.* Genetic polymorphism of chicken was investigated using PCR RFLP method. There were three stages of identification: extraction of DNA genom, PCR amplification of iNOS gene (with size 449 bp), and RFLP method using restriction enzym (*AluI*). The data were analyzed include of genotype and allele frequencies, heterozygosity values and Hardy-Weinberg equilibrium (HW). The result showed that iNOSgene was showed polymorphic in kampung chickens population. It were found two alleles (T and C ), and three genotypes (TT, TC and CC). The chi square test revealed the locus of iNOS was deviated in Hardy Weinberg equilibrium. Association analysis indicated that CC genotype was signifcantly associated ($P < 0.05$) withdisease resistance than TC and TT genotypes in Kampung chicken, that's mean TC and TT genotype has hight disease resistance than CC. In conclusion the polymorphism of chicken iNOS gene could be potential to the candidate gene in breeding programe for increasing genetic resistance against *S. pullorum*in kampung chickens.

Keywords: chicken, iNOS gene, *Salmonella pullorum*, Hardy-Weinberg (HW)

## 1. Introduction

Kampong Chicken is Indonesian native chickens, that it have been domesticated. It is different with other chicken, Kampong chicken does not have typical characteristic and spread the territory of Indonesia [1]. Local chicken, including kampong chicken, have a high resistance to virus infections [2]. Referensi[3]were reported kampong chicken resistance to *Salmonella enteritidis* infection.

*S. enteritidis* is one of *Salmonella*sp bacterium which are zoonotic. The S. enteritidis could be caused salmonellosis in humans who consume eggs by chickens that infection. The effect of *S. entritidis* is very different from the *S. pullorum*.

S. *pullorum* is pathogenic to chickens and can cause death [4]. S. pullorum bacteria can cause illness defecation lime. The disease attacks the digestive organs (Oliveira et al. 2000)[5].Once the importance of this disease, the government made a decree that all breeding chicken in Indonesia must be certified free from S. [6].

The resilience of the living body is influenced by genetic and environmental as well as the interaction of the two. There are several genes that control the body's resistance chicken, one of which is a gene inducible nitric oxide synthase (iNOS). INOS gene is a gene that synthesizes the iNOS protein. This protein acts as cytosolic enzymes, which catalyze L-arginine into nitric oxide (NO). NO is one of the reactive oxygen species that can act as cytotoxic compounds to kill bacteria that infect immune cells [7].

INOS gene in chickens located on chromosome 19. The structure of iNOS gene is promoter region, 28exon, 27 introns and flanking region with a length of 22.028 bp (Ensembl, NOS2 ENSGALG00000038096). Results of previous studies mentioned that iNOS gene is polymorphic in local chicken [8], on Broiler chickens [9] and at Leghorn chickens [10].

The purpose of this study is to identify the genotype diversity iNOS gene as controlling disease resistance in chicken and to test for association with disease resistance properties caused by the bacterium *S. pullorum*.

## 2. Material and Methods

The study was conducted from July to Oktober 2016. The research was conducted at Field Laboratory, Laboratory of Molecular Animal Breeding and Genetics, Department of Animal Production and Technology Science, Faculty of Animal Science, Bogor Agriculture University and Laboratory of Medical Microbiology, Faculty of Animal Veteryner Bogor Agriculture University. The number of samples were used 44 of chicken blood. For the association with body resistance in chicken were used 9 of blood samples that represent each genotype.

DNA extraction using procedure phenol-chloroform method [11], involves preparation samples, protein degradation, degradation of organic material, precipitation of DNA and DNA stored at -20 ° C.

The PCR amplification were used to amplify iNOS gene with length products 449 bp. Primer were designed using Primer Designing Tools Program. The PCR reactions were performed in 0.5 mL of chicken DNA sample, 10.85 mL DW, 0.3 mL of each primer, 0.05 mL of each Taq polymerase, 1.5 mL of ech buffer, 0.3 mL of each dNTP and MgCl 2 mL 1:00. The mixture is inserted into the PCR machine with the conditions of initial denaturation temperature of 95 ° C for 5 min, 35 cycles consisting of denaturation 95 ° C for 10 seconds, anaeling 60 ° C and 72 ° C final extention for 5 minutes.

RFLP method was used to determine the genotype iNOS gene. PCR result of the TLR4 gene fragment was cut by *AluI*restriction enzyme (0.3 mL) for 6 hours at 37 ° C. Visualized by electrophoresis using a 2% agarose gel with 0.5 TBE buffer (Tris Borate EDTA). The data were analyzed Allele frequencies, genotypic frequencies, Hardy-Weinberg equilibrium and heterozygosity.

The polymorphism of iNOS genes associated with body resistance traith, challenge testwith S. pullorum. Challenge testusing clearance test method [12].includes Bacteria Culture Preparation, chicken blood examination and interpretation of results.

The data were analyzed using *analysis of varian* (ANOVA) with completely randomized design. iNOS gene genotype was as treatment and observation data were as response.

$$Yij = \mu + Pi + \varepsilon ij \text{ [13]}$$

Where Yij is the trait observed on the genotipe, μ is the overall mean, Pi is effect of the single nucleotide polymorphism genotypes, and εij is the random residual effect.

## 3. Result andDiscussion

INOS gene in kampungchickens located on chromosome 19. The structure is promoter region, 28 exon, 27 introns and flanking region with a length of 22.028 bp (Ensembl, NOS2 ENSGALG00000038096). Allele frequencies and genotype frequencies iNOS gene in kampung chicken showed in Table 2.

The results showed indicate two alleles in iNOS gene, namely T and C allele which cutting by enzymes *AluI*. The size product PCR T allele of 310 bp and 139 bp and C allele of 449 bp.Frequency T and C allele in kampong chickens in this study respectively 0.602 and 0.398.

Table 1. Primer sequences, PCR product size for identification of polymorphism in Inos.

| Gene | Genbank accession No | Primer Sequence (5'-3') | SNP | PCR Product |
|---|---|---|---|---|
| INOS | NOS2 ENSGALG00000005693 | F:CCAAGGACTTACAGGTGTGG<br>R: CCAGGATGTTTGGGCTGTTG | Intron 21 (T→C) | 449 bp |

Tabel 2.The Genotypic and AlleleFrequencies of iNOS in KampungChickens.

| Chicken | N | Frekunsialel | | Frekuensigenotipe | | |
|---|---|---|---|---|---|---|
| | | T | C | TT | TC | CC |
| Kampong | 44 | 0.602 | 0.398 | 0.455 (20) | 0.295 (13) | 0.250(11) |

Tabel 3. The Observed, and ExpectedHeterozygosity for in KampungChickens.

| Chicken | N | He | Ho | $\chi^2$ |
|---|---|---|---|---|
| Kampung | 44 | 0.479 | 0.295 | 6.464 |

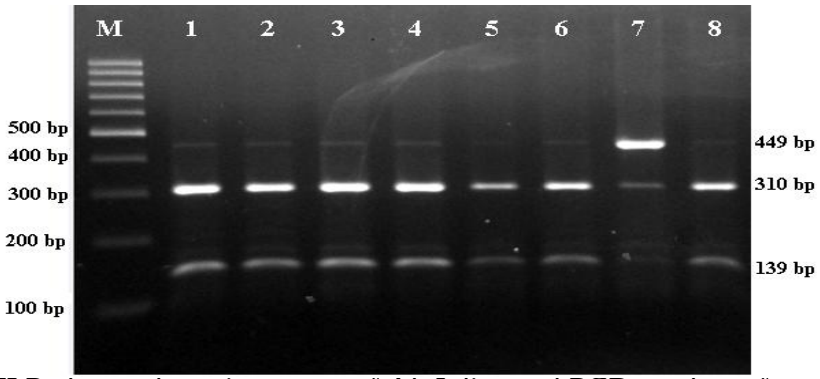


Fig. 1 PCR-RFLP electrophoresis pattern of AluI digested PCR products from Inos gene with 2% agarose gel

Table 4. Association BetweeniNOSGeneAluI with ResistanceAgaint to *Salmonellapullorum*

| Genotype INOS gene | First Consentration (cfu/ml) | Final Konsentration (cfu/ml) | The Mortality Rate of Bacteria (%) |
|---|---|---|---|
| TT | $6.8 \times 10^{10}$ | $4.96 \times 10^{8}$ | $99.516^{a}$ |
| TC | $6.8 \times 10^{10}$ | $2.87 \times 10^{8}$ | $99.830^{a}$ |
| CC | $6.8 \times 10^{10}$ | $7.4 \times 10^{8}$ | $98.910^{b}$ |

Same latter means in the same column with different superscripts differ signifcantly (P<0.05).

Mutation of the allele T / C on the fragment to 310 bp, it is consistent with the statement referensi[8] that there is a mutation of the allele T / C similar to that reported previously that the digested fragments are due to a T/C substitution at position 173 bp in intronic region for iNOS.

In the iNOS gene was founding three genotypes namely TT, TC and CC (figure 1). Genotype frequencies was obtained TT (0.455), TC (0.295) and CC (0.250). the result genotyping iNOS gene in kampong chicken locus *AluI* has polymorphic. According to referensi[8]that iNOSgene has polymorphic at Malaysia native chicken.

**Heterozigosity Dan Genotype EquilibriumiNOSGene**

Observed and expected genotypic and allele frequencies for the the candidate genes are displayed in Table 3. The observed and expected heterozygosity for iNOS was 0.479 and 0.295. Heterozygosity value is the average percentage of heterozygous loci per individual or the average percentage of heterozygous individuals in the population [14].

Genotype equilibrium iNOS gene in the population (Hardy-Weinberg equilibrium), were analyzed by chi-square ($\chi^2$). The test results of gene iNOS on *AluI* locus can be seen in Table 3. The chi square test revealed the locus of iNOS was deviated in Hardy Weinberg equilibrium.

Association of iNOS gene genotype and the expression of resistance against *S. pullorum* infection in Kampong chicken

Polymorphism OF Inosgene were associated with resistencetraith to S. pullorum bacterium in kampong chicken. Challenge test: was used clearance test method [12].Association between iNOS gene AluI with resistance againt to salmonella pullorum represented in table 4.

The clearance test to *S.pullorum* in kampung chicken showed TT, TC and CC genotype can be killed the S. pullorumbacterium. The clearance test showed 99.516% at TT genotype, 99.830% at TC genotype and 98.910% at CC genotype. Acording to Statistical analysis, mortality of bacterium level was Associated significantly with genotype of iNOS gene. Genotipeof TT and TC was higher than CC genotype. Referensi[10], reported that Inos gene were associated with response to *S.enteritidis* in leghorn chicken.

**Conclusion**

This study concluded that TT and TC genotype was signifcantly associated ($P < 0.05$) with disease resistance than CC genotypes in Kampung chicken. Polymorphism of chicken iNOS gene could be potential to the candidate gene in breeding programe for increasing genetic resistance against *S. pullorum*in kampung chickens.

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**References**

[1] Nataamijaya AG, Pengembangan potensi ayam local untuk menunjang peningkatan kesejahteraan petani. *J Litbang Pertanian*.29(4):131-138. 2010.

[2] Maeda, Polymorphism of Mx gene in Asia indigenous chicken population. Makalahdipresentasikanpada Seminar NasionalTentangUnggasLokal III.UniversitasDiponegoro. 25 Agustus 2005.

[3] Ulupi N, Muladno, C Sumantri, I.W.T Wibawan,. Study of kampung chicken resistance against *Salmonella enteritidis* using TLR4 gene as marker. *IJPS*. 13:467-472. 2014.

[4] OIE (Office International des Epizooties), Fowl typhoid and pullorum disease. OIE Terrestrial Manual, Office International des Epizooties, Paris, France, 2008.

[5] Oliveira G. H., J. A Berchieri.and P. A Barrow, Prevention of *Salmonella* Infection by Contact Using Intestinal Flora of Adults Birds and/or a Mixture of Organic Acids. *Braz. J. Microbiol*., 31: 116-120. 2000.

[6] [BSN] BadanStandarisasiNasional. SNI 01-4868.2-2005.SyaratBibitNiaga. BadanStandarisasiNasional. Jakarta, 2005.

[7] Wei XQ, Charles IG Smith, A, Ure J, FengGj, Huang FP, Xu D, Muller W, Moncad S. &Liew FY. Altered immuneresponses in mice lacking inducible nitric oxide synthase.*Nature*. 375:408-411, 1995.

[8] Tohidi R, Idris I, Panandam JM danBejo MH. The Effects Of Polymorphisms In IL-2, IFN-G, TGF-B2, Igl,TLR-4, MD-2, And iNOS Genes On Resistance To *Salmonella enteritidis*In Indigenous Chickens. *Avian Pathology*. 41(6): 605-612. 2012.

[9] Kramer J, Malek M. & Lamont SJ. Association of twelve candidate gene polymorphisms and response to challenge with *Salmonella enteritidis* in poultry.*Animal Genetics*.34 : 339 - 348. 2003.

[10] Malek M, Hasenstein JR, Lamont SJ. Association of *iNOS*, *TRAIL*, *TGF-b2*, *TGF-b3*, and *IgL*genes with response to *Salmonella enteritidis*in poultry.*Poult. Sci.* 83: 544-549. 2003.

[11] Sambrook J, Fritsch EF, Maniatis T. *Molecular Cloning: A Laboratory Manual*. (US): CSH Laboratory Pr. 1989.

[12] George JJ. 1998. *Bacteriogical Analytical Manual*. Published and Distributed by AOAC International.

[13] Mattjik AA, Sumertajaya M. *PerancanganPercobaandenganAplikasi SAS dan Minitab.* Ed ke-2.Bogor (ID): IPB Press. . 2002.

[14] Nei M, Kumar S. *Molecular Evolution and Phylogenetics*. New York (US): Oxford Univ Pr. 2000.

# Investigation of Cadmium Contamination in Mealworm, Ration and Broilers's Feces

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## Abstract

Mealworn (*Tenebrio molitor L*) is a beetle larva that can be used as a source of protein a substitute for MBM. Cadmium is one of heavy metals that disturb metabolic process leading to toxicity to the broiler. As far as the feed ingredients is anintegral part of the consumer's food chain, it's need to be assessed as potential sources of Cd contamination. However, a study about utilization mealworm in broilers feed formulations has been done yet in the point of viewed of food safety, especially Cadmium (Cd) free.The objective of this research was to investigate the contamination levels of cadmium in mealworm, ration and broiler's feces. A total 200 DOC (Lohman strain) placed in 20 plots devided into two treatments, T0 was broilers fed with 0% mealworm concentrate, 5% MBM and T1 was 5% mealworm concentrate, 0% MBM. The Cd contamination in the mealworm, ration (T0 and T1), and broiler's feses were analyzed using ICP-OES instrument method. The result showed that no Cd contamination were detected in the mealworm, ration and broiler's feces from both tratments. It could be conclude that the mealworm is a safe ingreedient for feed.

**Keywords**: mealworm, cadmium, broilers, ration, feces

## 1. Introduction

Feed is one of the factors that having an important role in theraising of broilers. A qualified feed has a complete composition of substances, such as proteins. Especially the protein source derived from animal are commonly used in poultry feed mixture in many countries such as *Meat and bone meal* (MBM), this is used as a source of amino acids, calcium (Ca) and phosphorus (P) [1]. Until now, Indonesia, still importing about100% of MBM from Australia, New Zealand and America. The Efforts to overcome the issue was using feed based raw materials local a substitute for mbm are insects (mealworm).

The mealworm with latin name's *Tenebrio molitor L*, is the larva of the beetle rice [2]. Mealworms can be used as a substitute protein source MBM, because it is local feed ingredients, high nutrient density, easily maintained, high production, and environmentally friendly. However, no studies using mealwormas a subtitute of MBM in broiler feed formulations in the point of viewed of food safety. Before being introduced as a new raw material for animal feed, it's need to do further research on food security because the insect was suspected containing harmful chemicals. Some of these chemicals can be present in the substrate of

insects, especially heavy metals, namely cadmium.

Reference [3] shows cadmium is the most toxic among other heavy metals in the water and soil. Total production of cadmium in the worldwide is estimated about 22,300 tons according to the British Geological Survey in 2010. Cadmium is heavy metal toxic highly dangerous for human and other mammals. Cadmiun contamitation is from the air, soil, water and smoke. After food contamined cadmium be in the body, it can be accumulated in several organs and tissues, including in the liver and kidneys [4]. The exposure of cadmium caused harmful effects on health, including renal dysfunction, heart disease, hypertension, osteoporosis, liver toxicity, changes in the activity of the pancreas and cancer [5]. Cadmium wasclassified as carcinogenic material to humans and animals [6].Itai-itai disease is a bone disease caused by chronic cadmium poisoning, this occurred in Japan in the food and water supply ndustri in the river Jinzu [7].

Previous studies showed that products derived from animals, food and feed products derived from insects were suspected having hazardous chemicals, by environmental contaminants, for instance heavy metals [8]. According to [10],in the mealworm *(T. molitor* ) was found cadmium (Cd)thatwere maintained in several different soil characteristics. In agreemnet to [9] that insects can accumulate cadmium. Investigation of cadmium level in the mealworm would be very importance, since mealworm is local ingridient that subtitute MBM with high protein content. Therefore, the objective of this study was investigating Cd contamination in mealworms, broiler's rations containing flour mealworm and feces of broiler that was fed with ration containing flour mealworm.

## 2. Material and Methods

2.1. Treatments

A total of 200 DOC *(Day Old Chick)* MB 202 Platinum *(Lohman strain)* from PT JAPFA Comfeed Indonesia Tbk were raised in the cages until 35 days old, then its was moved into the induvidual cage until38 daysold. All the broilers were divided into two treatments and 10 replications. T0: mealworm ration containing 0%, 5% MBM (control), R1: mealworm ration containing 5%, 0% MBM. Ration and drink were given by ad libitum.

2.2 Sample collection

The mealworms aged 2-4 month were obtained from several large farmers in Indonesia, such as Malang, Bekasi and Bogor. All samples were mixed into a composite sample, then mealwormswere processed into mealworm concentrate, and then were formulated with other feed such as corn, soybean meal, rice bran, palm oil, limestone, salt, DL-Methionin 99%, DCP (P18%) to be ration T1, as well as additional rations T0 with MBM and other feed. Samples of ration were taken after the process of formulating finisher ration.

2.3 Analysis of Samples

Cadmium contamination in all samples (2-4 month of ) were measured according to [11]. In Brief, [11] explained that the samples were processed in $HNO_3$ to detect Cadmium and were analyzed using *inductively coupled plasma optical spectrometry* (ICP OES, Agilent type CCD detector 720, USA). As much as 0.5 ml sample were added 10 ml $HNO_3$, destruction for 15 minutes at temperature of $150^0$ C. After that samples were put into the 40 ml flask and diluted with aquabidest and filtered. Analysis was conducted using *inductively coupled plasma optical spectrometry* (ICP OES) with a wavelength of 214 439 nm cadmium.Calibration curve was analyzed to calculate the levels of cadmium contamination. All of analysis was carried out by duplo.

2.4 Data Analysis

Cadmium contamination in mealworm, mealworm concentrate, ration and feces were observed. If any contamination was found in the feses, then proceed to the analysis of the liver and kidneys contamination. The data was analysis using descriptive method by comparing Cd levels in the mealworm, ration and feces with the applicable standards.

## 3. Result and Discussion

3.1 Cadmium contamination in mealworm

The analysis of mealworms that were obtained from several regions showed no cadmium contamination was detected. Reference [9] showsmaximum limit of cadmium metals in the feed material derived from animal is 2 mg/kg or 2 ppm (88% dry matter). In general, farmers in Indonesia maintains mealworm intensively, in a box plywood boards are arranged in a closed room, with the provision of feed substrate is maintained such as pollard and bran. This evidence showed the source of feed, mealworms environment is also not detected cadmium.

Demand [12] stated there are a few studies explore about the absorbed mechanism ofmetal in terrestrial invertebrates, and this study mostly done on earthworm. Heavy metal contamination on a mealworm through consumption of contaminated food [10]. Its is different from the earthworm, metal contaminants enter the body through the skin.This is because the mealworm has the cuticle layer of wax that serves to avoid water loss, so that the metal contamination does not easily go in through the body, but the food, in contrast to earthworms that have skin that can be penetrated by water and metal contamination [10]. Reference [13] shows found that the larvae of the mealworm able to control contamination of cadmium in the body when exposed to metal contamination through the feed. They also found that most of the accumulation of cadmium may be removed during metamorphosis. Reference [14] shows that cadmium can form a soluble inorganic lipid compound that can easily pass through microvilli of the gastrointestinal tract of animals, in which the walls of the gastrointestinal tract is the main organ for the stack of metal contamination.

3.2 Cadmium contamination in the ration

Table 1 showed that contamination level of Cd in all samples. All of samples were detected have no contamination of Cd. Reference [15] shows limit of cadmium in the diet about 0.5 ppm according to EU standards. The concentration of cadmium in food is influenced by food type and geographic region. Cadmium can be obtained from plants and animals [7]. Cadmium with low concentrations is not toxic, but when accumulated to a certain degree can be toxic to animals or humans pass through the food chain [16]. According to the FAO or WHO, consumtion of Cd per week which is tolerable for humans is 400-500 ug/person or 7ug/kg of body weight (0,007 ppm) [17]. Meanwhile, that cadmium toxicity lethal at a dose of 225 ppm consumption, with a tolerable weekly intake is 0.007 ppm weight (p*rovisional tolerable weekly intake / PTWI)* [18].

3.3 Cadmium contamination in the feces

In table 1 the T0 and T1 treatment there are no cadmium contamination. T0 and TI in-

Tabel 1. Cadmium Contents Ration and Feces

| | T0 | T1 | Limit of Detection ICP-OES *(ppm)* | Literature |
|---|---|---|---|---|
| Ration | Nd | Nd | 0.00011 | 0,5 ppm [15] |
| Feces | Nd | Nd | 0.00011 | Cd contents in animal feeds: 0,851 ppm, Cd contents in manure: 1,281 ppm [11] |

Information: Nd= Not detected

dicated that the rations, feces water and a safe environment from cadmium. No detection of cadmium states that the study was conducted in a good environment. Reference [11] shows states that the average cadmium contamination in livestock rations 0.851 ppm, and the manure became 1.281 ppm, the addition of supplements allegedly occurred outside the ration and environmental factors close to traffic and industrial pollution.

## Conclusion

This result showed that no Cd contamination were detected in the mealworm, ration and broiler's feces from both tratments. So, it could be conclude that the mealworm is a safe ingreedient for broiler feed and could be utilize as a subtitute of MBM.

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## References

1. Mutucumarana RK, Ravindran V,"Measurement of true ileal phosphorus digestibility in meat and bone meal for broiler chickens using the direct method". *Animal Feed Science and Technology*., 219:249–256. 2016.
2. Ramos Elorduy J, Gonzalez EA, Hernandez AR and Pino JM, "Use of Tenebrio molitor (Coleoptere: Tenebrionidae) to recycle organic wastes and as feed for broiler chickens".,*J Economic Entomology.* 95:214–220. 2002.
3. M. Khairy, S.A. El-Safty, M.A. Shenashen, "Environmental remediation and monitoring of cadmium, Trend Anal". Chem. 62:56–68. 2014.
4. Järup L, Akesson A,"Current status of cadmium as an environmental health problem". Toxicol Appl Pharmacol.,238(3): 201-208. 2009.
5. Donpunha W, Kukongviriyapan U, Sompamit K, Pakdeechote P, Kukongviriyapan V, Pannangpetch P, "Protective effect of ascorbic acid on cadmium-induced hypertension and vascular dysfunction in mice". Biometals 24(1):105-115.2011
6. IARC. Monographs on Evaluation of câncer risk to humans,1993.
7. Zang Y, Bolger PM, "Toxic metals: cadmium. Reference module in food science". Encyclopedia of food safety. 2:346–348. 2014
8. Zhuang P, Zou H and Shu W, "Biotransfer of heavy metals along a soil-plant-insect-chicken food chain: Field study". *J Environmental Sciences*. 21:849–853. 2009.
9. Charlton AJ, Dickinson M, Wakefield ME, Fitches E, Kenis M, Han R, Zhu F, Kone N, Grant M, Devic E, Bruggeman G, Prior R and Smith R, "Exploring the chemical safety of fly larvae as a source of protein for animal feed". *Journal of Insects as Food and Feed*. 1(1): 7–16.2015.
10. Vijver M, Jager T, Posthuma L and Peijnenburg W, "Metal uptake from soils and soil–sediment mixtures by larvae of Tenebrio molitor (L.) (Coleoptera)". *J Ecotoxic and Environmental Safety*. 54:277–289. 2003.
11. Wang H, Dong Y, Yang Y, Gurpal S. Toor, Zhang X. "Changes in heavy metal contents in animal feeds and manures in an intensive animal production region of China". *Journal of Environmental Sciences*. 25(12): 2435–2442. 2013.
12. Beaumella, L., Gimbert, F., Hedde, M, Guérin,A.,Lamy,I.,"Subcellular partition in gofmetalsinAporrec to deacaliginosa along agradient of metal exposure in 31 field-contaminated soils".Sci.TotalEnviron.520,136–145. 2015.
13. Lindqvist L, Block M,"Excretion of cadmium during moulting and metamorphosis in Tenebrio molitor (Coleoptera; Tenebrionidae)". *CompBiochem. Physiol*. C 11:325–328. 1995.
14. Pedersen SA, Kristiansen E, Andersen RA, Zachariassen KE, "Cadmium is deposited in the gut content of larvae of the beetle Tenebrio molitor and involves a Cd-binding protein of the low cysteine type". *Comparative Biochemistry and Physiology*. Part C 148:217–222. 2008.
15. EFSA Scientific Committe,. "Scientific Opinion on a risk profile related to production and consumtion of insects as food and feed". EFSA Journal,. 13(10):4257,60pp.2015.
16. Miller, T.G, Jr, Living in The Environment : Principle, Connection and Solutions. Singapore: Thompson Brooks/Cole. 2007.
17. Lide DR,*CRC handbook of chemistry and physics*. 86th ed. Boca Raton (FL): CRC Press. p. 4-80. ISBN 0-8493-0486-5.2005.
18. ISN (Indonesian Standard National), The maximum limit of heavy metal contamination in food. SNI 7387. 2009.

# Malonaldehyde and Fat Contents of Kampong-meat Type Crossbreed Chicken

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## Abstract

Malonaldehyde (MDA) is a toxic and mutagenic compund produced from lipid oxidation, and also correlated to the rancid flavorof products.The occurence of MDA in meat poultry and its productsshould be controled as low as possible. The objective ofthis study was to reduce MDA content in meat chicken through crossing between kampong and meat type chicken, and evaluate their fat content.A number of 30 chickens were devided into 5 groups including: 1) meat type chicken (parent stock Cobb Strain) (MTC); 2) Kampong chicken (KC); 3)F2 Kampong-meat typecrosbreed chicken fast growth (KMCFG); 4) F2 Kampong-meat typecrossbreedchicken medium growth(KMCMG);5) F2 Kampong-meat typecrossbreed chicken slow growth (KMCSG). Samplesused were meat from thigh part without skin. Each group contained 3 heads of male and 3 heads of female chickens.MDA content was estimatedby thiobarbituric acid reactive substances value. Male crossbreed chickens contained MDA lower than KC($p<0.05$) and no significant different with MTC. Female KMCFG and KMCMG contained MDA lower than KC ($p<0.05$) and not different with MTC. KMCMGcontained MDA level not different withKC and MTC. All male group had no different fat content, but female group showed that KChad higher fat content than MTC ($p<0.05$). Fat content of KMCSG was not different withKCorMTC. Fat content of KMCMG and KMCFG were not different with fat content of KC.In conclusion, crossing meat between KC and MTCreduced MDA content for all groupF2 chicken, except female F2 KMCMG, but not with fat content.

Keywords : Malonaldehyde, fat, Kampong-meat type crossbreed chicken

## 1. Introduction

Kampong chicken is an original chicken, which has been adapted to the tropical environment of Indonesia (Iskandar, 2010). Kampong chicken has some potential, such as the high number of the genetic and phenotypic diversity, high levels of adaptation, heat resistance, and disease resistance. (Nataamijaya 2000; Mardiningsih et al., 2004; Pagala et al., 2013; Tamzil et al., 2013; Ulupi et al. 2013). As a producer of meat, Kampong chicken has commercial potential to be developed, because the meat is highly favored by several people in Indonesia.

Productivity improvement efforts is not enough just with increased of feed quality and maintenance management, but need to increase genetic quality also by breeding programs. One of the breeding program, heading to increase productivity could be done by crossbreeding. This crossing was expected to be increase the average body weight and slaughter weight of chicken rapidly in short time (Kgwatalala*et al*. 2015).

Crosses could be done with meat-type chicken (broiler) to incerase the results from a combination of two clusters of the chicken.

Meat quality is the major priority consumers to select chicken meat for consumption. One of the factors that influence the quality of the meat is fatty acid oxidation.Hight rate of fatty acids oxidation reduces the quality of meat and meat products, negatively affecting their flavor, odor, color, and texture. (Avila-Ramos *et al*. 2013). MDA is a secondary product of lipid oxidationthat hascontribution to the off-quality of meat product (Fernandez, Perez-Alvarez, & Fernandez-Lopez, 1997). The high level of MDA content could be potent ashazard in meat and meat product as a result of lipid oxidation in meat (Suryati*et al*. 2013). This study aimed to reduce MDA content in meat chicken through crossing between kampong and meat type chicken, and evaluate their fat content.

## 2. Material and Methods

*2.1 Materials*

A number of 30 chickens were devided into 5 groups including: 1) meat type chicken (parent stock Cobb Strain) (MTC); 2) Kampong chicken (KC); 3)F2 Kampong-meat typecrosbreed chicken fast growth (KMCFG); 4) F2 Kampong-meat typecrossbreedchicken medium growth(KMCMG);5) F2 Kampong-meat typecrossbreed chicken slow growth (KMCSG). Samplesused were meat from thigh part (drumstick) without skin. Each group contained 3 heads of male and 3 heads of female chickens. Analyses wereconducted by using chemicals of analytical grade: thiobarbituric acid (TBA) from Merck (Merck KGaA, Germany), and 1,1-diphenyl-2picrylhydrazil (DPPH), propylgallate (PG), ethylenediaminetetraacetic acid (EDTA), 1,1,3,3-tetraethoxypropane (TEP), 1-(N)naphthylethylenediaminedihydrochloride and sulfanilamide from Sigma (Sigma Aldrich Co., USA).

*Malonaldehyde (MDA) Analysis*

Malonaldehydecontentwas determinedby using thiobarbituric acid reactive substances (TBARS) analysis according to the method as described by Sørensen and Jørgensen (1996)with a little modification. The modification was the homogenization of sample before the addition of PG and EDTA solution (Suryati et al. 2013). TBARS analysis by spectrophotometer (GeneQuant 1300, Sweden) was done after 5 mL of sampledistillate was reacted with 5 mL TBA 0.02 M and then incubated at 100°C for 40 min. Absorbance at λ 532 nm was measured using two replications for each sample. TBARS was expressed as mg of malonaldehyde (MDA) per kg dry matter (DM) of dendeng using TEP as a standard.

*Fat Content Analysis*

Fat content was analysed according to themethod described by AOAC (2005).Fat content was expressed as percent (%) from sample wei

## 3. Result and Discussion

*3.1 Malonaldehyde (MDA)Content*

The MDA content of Kampong, meat-type, Kampong-meat type crossbreed chickens with different rate growth are presented in Table 1.Male crossbreed chickens contained MDA lower than Kampong chicken ($p<0.05$) and no significant different with meat-type.Female crossbreed fast and slow growth contained MDA level lower than Kampong chicken ($p<0.05$) and not different with meat type chicken as their parent. Kampung-meat type crossbreed medium growth contained MDA level not different both of Kampung and meat type chickens. Statistically MDA level of Kampong Chicken was not different with MDA level of meat type chicken.

Table 1. Malonaldehyde (MDA) Kampong, meat-type, Kampong-meat type crossbreed chickens with different rate growth

| Chicken types | Sex | |
|---|---|---|
| | Male | Female |
| Kampong | 4.06±2.59[a] | 5.63±3.82[a] |
| Meat-Type | 1.52±1.25[b] | 1.95±1.09[ab] |
| Kampong-Meat type crossbreed fast growth | 1.33±0.44[b] | 1.52±0.79[b] |
| Kampong-Meat type crossbreed medium growth | 0.78±0.26[b] | 2.02±0.39[ab] |
| Kampong-Meat type crossbreed slow growth | 1.24±0.35[b] | 1.18±1.61[b] |

Note: Different superscriptsin the same column mean significant different (p<0.05).

Table 2. Fat content of Kampong, meat-type, Kampong-meat type crossbreed chickens with different rate growth (%)

| Chicken types | Sex | |
|---|---|---|
| | Male | Female |
| Kampong | 3.03±0.83 | 6.76±1.57[a] |
| MeatType | 4.26±0.83 | 2.03±1.68[b] |
| Kampong-Meat type crossbreed fast growth | 4.65±1.48 | 5.19±1.06[a] |
| Kampong-Meat type crossbreed medium growth | 2.88±1.71 | 4.76±1.62[a] |
| Kampong-Meat type crossbreed slow growth | 4.07±1.05 | 4.40±0.94[ab] |

Note: Different superscriptsin the same column mean significant different (p<0.05).

Frozen storage could affect the MDA content drumstick chicken meat. The mean concentrations of MDA increased 44% in fat from both breast and leg meat after 3 months of frozen storage and increased 2.5 and 2.2 times in fat from breast and leg after 6 months of frozen storage (Abdel-Kader 1996). The MDA content could affect by stress also, such as heat stress or stress before kill. Heat stress increased lipid peroxidation as a consequence of increased free radical generation, as indicated by MDA concentration (Altan*et al* 2010). Heat stress also caused oxidative stress, increased red blood cell susceptibility to peroxidation,as indicated by increased MDA concentration (Altan*et al* 2010).

*3.2 Fat content*

The Fat content of Kampong, meat-type, Kampong-meat type crossbreed chickens with different rate growth are presented in Table 2. Male group for all group had no different fat content, but female group showed that Kampong chickens contain fat higher than meat type chickens ($p < 0.05$). Fat content of Kampong-meat type crossbreed slow growth was not different with either Kampong nor meat type chicken. Fat content of Kampong-meat type crossbreed medium growth and Kampong-meat type crossbreed fast growth were not different with fat content of Kampong chicken.

The present feed chicken by adlibitum could affect the fat content of chicken. Fat content in the body animal is obtained from advantages energy consumed. Food rations consumed by the excess energy are saved as fat, so that the higher energy content of the ration, the higher the fat content in the body (Anggorodi 1985).

The results of chicken meat by fat content included normal, ranged between 1.2% to 12% (Aberle et al., 2001). The fat content of meat is influenced by breed, location of muscle, muscle type, sex and age of animal. The percentage of fat in general increases with age but can be changed at any time depending on the nutrients consumed

(Soeparno 1994), according Aberle et al, (2001) fat content meat varies depending on the amount of external fat and intramuscular fat.

## Conclusion

Crossing meat between KC and MTC reduced MDA content for all group F2 chicken, except female F2 KMCMG, but not with fat content.

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## References

Aberle, E. D., Forest, C. J., Hedrick, H. B., Judge, M. D., and Merkel, R. A. 2001. The Principle of meat science, W. H, Freeman and Co. San Fransisco,

Altan Ö, Pabuccuoglu, A., Altan, Konyalioglu, S., and Bayraktar, H. 2010. Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. Department of Animal Science, Ege University Faculty of Agriculture, Bornova, Izmir, Turkey.

AOAC. 2005. Official methods of analysis of AOAC International (18th ed.). AOACInternational. Maryland.

Avila-Ramos, F, Pro-Martínez, A., Sosa-Montes, E., Manuel, J., Cuca-García, Becerril-Pérez, C., Figueroa-Velasco, J. L., Ruiz-Feria, C. A., Hernández-Cázares, A. S., and Narciso-Gaytán, C. 2013. Dietary supplemented and meat-added antioxidants effect on the lipid oxidative stability of refrigerated and frozen cooked chicken meat. Poultry Science, 92, 243–249.

Fernandez, J., Perez-Alvarez, J. A., and Fernandez-Lopez, J. A. 1997. Thiobarbituric acid test for monitoring lipid oxidation in meat. Food Chemistry, 59, 345–353.

Kgwatalala, P. M., Segokgo, P., and Simon, E. 2015. Comparative growth performance of cross-bred (50% orpington: 25% australorp: 25% tswana) and pure-bred tswana chickens under an intensive management system. Journal of Poultry Science, 14, 63-66.

Mardiningsih, D., Rahayuning, T. M., Roessali, W., and Sriyanto, D. J. 2004. Tingkat produktivitas dan faktor-faktor yang mempengaruhi tenaga kerja wanita pada peternakan ayam lokal intensif di Kecamatan Ampel Gading Kabupaten Pemalang Jawa Tengah. Seminar Nasional Teknologi Peternakan.

Nataamijaya, A. G. 2000. The Kampong chicken of Indonesia. *Buletin Plasma Nutfah*, 6.

Pagala, M. A., Muladno, Sumantri, C., and Murtini, S. 2013. Association of mx gene genotype with antiviral and production traits in Tolaki Chicken. International Journal of Poultry Science. 12, 735-739.

Soeparno. 1994. Ilmu dan teknologi daging. Gadjah Mada University Press, Yogyakarta.

Suryati, T., Astawan, M., Lioe, H. N., and Wresdiyati, T., and Usmiati. 2013. Nitrite residue and malonaldehyde reduction in dendeng—Indonesian dried meat — influenced by spices, curing methods and precooking preparation. Meat Science, 96, 1403–1408.

Tamzil, M. H., Noor, R. R., Hardjosworo, P. S., Manalu, W., and Sumantri, C. 2013. Acute heat stress responses of three lines of chickens with different Heat Shoc Protein (HSP) 70 genotypes. International Journal of Poultry Science. 12, 264-272.

Ulupi, N., Muladno, Sumantri, C., and Wibawan, I. W. T. 2013. Association of TLR4 gene genotype and resistance against *Salmonella enteritidis* natural infection in kampung chicken. International Journal of Poultry Science. 12, 445-450.

# Quality of Gelatin Processed from Chicken Legs (*Tarsometa tarsus*) Skin with Different Method

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## Abstract

This study aimed to utilize chicken legs skin (*Tarsometa tarsus*) to produce fine quality and physicochemical properties of gelatin derived from halal ingredients by using different degreasing methods of processing. Methods used in gelatin production were: 1) skin sample is not soaked in NaOH 0.05 M and liquid gelatin is centrifuged for 15 min with 3500 rpm (WONS); 2) skin sample is not soaked in NaOH 0.05 M and liquid gelatin is centrifuged for 15 min with 3500 rpm and then ethanol is added (1:1) (WONSE); 3) skin sample is not soaked in NaOH 0.05 M ethanol is added to liquid gelatin (1:1) (WONE); 4) skin is soaked in NaOH 0.05 M and liquid gelatin is centrifuged for 15 min with 3500 rpm (WNS); 5) skin is soaked in NaOH 0.05 M and liquid gelatin is centrifuged for 15 min and then ethanol is added to it (1:1) (WNSE); 6) skin is soaked in NaOH 0.05 M and ethanol is added to liquid gelatin (1:1)(WNE). Results showed that the different methods significantly affected ($p<0.05$) pH, viscosity, intensity of yellow (b value), protein and fat contents. WONS method produced the highest viscosity ($p<0.05$) and protein content ($p<0.05$), as well as the lowest fat content ($p<0.05$) with no differences for other characteristics with other treatments. Therefore, it can be concluded that methods where the skin has not been soaked in NaOH 0.05M and liquid gelatin has been centrifuged are the best ones to produce fine quality gelatin from chicken legs skin.

Keywords: chicken skin, gelatin, quality

## 1. Introduction

Gelatin is produced by protein hydrolysis of bone collagen and skin, which are widely used for various industrial purposes, either food industries or non-food industries. The main functions of gelatin in the food industry are foaming stabilizer, gelling, adhesive, viscosity agent, and emulsifier. Usually, halal gelatin is processed from cattle bone and skin collagen. Nonetheless, chicken bone and skin collagen have potential use as sources of gelatin.

Chicken legs *(Tarsometa tarsus)* which is also called chicken claw has a high protein content, about 22% [16].The high content of protein in the skin, especially chicken legs collagen protein, can be used as an alternative to halal gelatin products that are consumed by most people in Indonesia, both Islamic and Hindu [5]. Broiler numbers in 2014 reached 1.443349 billion of head/year [6]. Based on this data, if a chicken weights 1.5 kg/head, then 2 165 023.5 tons of chicken/year would be produced. It is known that the weight of the chicken legs skin represents about 1% of

total body weight, therefore, in one year about 21 650.235 tons of chicken legs skin could be produced. It is indicated that chicken legs skin could have a good potential as a source of halal gelatin.

Chicken legs skin gelatin is still very high in fat. Fat content should not exceed 5%, as it is an important quality requirement for gelatin. The fat contained in gelatin can lead to fat oxidation which causes rancidity and shortens shelf life. Based on [12] report which uses skin gelatin produced from chicken legs, fat content of produced gelatin is usually 15.7%. Therefore, this study aimed to utilize the chicken legs skin *(Tarsometa tarsus)* to produce fine quality and physicochemical properties of gelatin derived from halal ingredients processed using different degreasing methods.

## 2. Materials and Methods

Extraction of gelatin

Chicken legs were taken from a slaughterhouse that already have halal standards. Boneless chicken legs skin and nails were first cleaned. Samples of skin were soaked in 0.05M NaOH for 1 hr (every 30 minutes NaOH was changed) and then were washed until the pH became neutral. The skin samples that where not soaked in NaOH were washed and then used directly in the next step. The skin samples were then soaked in a 1.5% HCl solution for 24 hours, and then washed until the pH became neutral. The skin samples with a neutral pH were then heated using a water bath at a temperature of 90$^{o}$C for 2 hours. The gelatin solutions that resulted were treated as follows: a gelatin solution was centrifuged at 3500 rpm for 15 minutes, a gelatin solution was centrifuged at 3500 rpm for 15 minutes and then ethanol 1:1 was added, and finally ethanol 1:1 was added to a gelatin solution. All samples were then dried using an oven at a temperature of 60$^{o}$C for 24 hr. The resulting gelatin was analyzed for its proximate content, viscosity, pH, yield, and color.

Proximate Analysis [1]

Proximate analysis of the chicken leg skin gelatin included moisture content, ash content, protein content, fat content.

Analysis of gelatin viscosity [8]

Gelatin solution (150 ml) with a concentration of 6.67% at a temperature of 60 °C was inserted into the space provided. The rotor was dipped into the sample and allowed to spin until the appointment of a scale needle stops at a certain scale. The scale read indicated the viscosity of the sample (cP).

Determination of pH [1]

Gelatin solution at concentration of 6.67% was placed into beaker glass. After calibration, cathode of pH meter was dipped in gelatin. The constant digital number displayed in the screen was pH value of sample.. The cathode was rinsed and dried for next measurement.

Determination of gelatin yield [11]

Yield determination was calculated based on the weight ratio of gelatin obtained against severe skin chicken legs, and expressed in percent (%).

Color Measurement [9]

Color analysis was performed using chromameter measuring the spectrum of light reflected from gelatin, determined by the coordinates L, a * and b *. Notation L *, 0 (black), 100 (white) showed reflected light producing achromatic colors of black gray and white.

## 3. Results and Discussion

The water content of the resulted gelatin meet standards set BSNI 01-3735-1995 (maximum 16%). Different fat removal methods showed no significant effects on moisture content, and resulted in low moisture content, since all samples received same extraction and drying methods.[2]stated that low moisture content in gelatin was due to the

Table 1. Proximate analysis of chicken leg *(Tarsometa tarsus)* skin gelatin with different fat removal methods

| Variables (%) | Treatments | | | | | | SNI |
|---|---|---|---|---|---|---|---|
| | WONS | WONSE | WONE | WNS | WNSE | WNE | |
| Moisture | 7.65± 1.79 | 7.68± 1.03 | 7.56±1.19 | 6.46± 0.75 | 6.99±0.38 | 6.69±0.53 | max 16% |
| Ash | 0.27±0.11 | 0.27±0.05 | 0.29± 0.07 | 0.29± 0.02 | 0.24±0.01 | 0.22±0.024 | max 3.25% |
| Protein | 92.36±0.35cd | 93.52±0.34bc | 89.22± 2.39d | 97.20±0.74a | 95.85±1.03ab | 91.35±2.74cd | |
| Fat | 0.92± 0.34 bc | 1.90± 0.49ab | 2.95 ± 0.59a | 0.45± 0.23 c | 1.62 ±0.91b | 2.81 ±0.49a | |

Different letters following the values in the same line indicate significantly different (P <0.05)

Notes: wons = skin without soaked NaOH and gelatin solution centrifuged, WONSE = skin without soaked NaOH and gelatin solution centrifuged and added ethanol, Wone = skin without soaked NaOH and gelatin solution was added ethanol, WNS = skin soaked NaOH and gelatin solution were centrifuged, WNSE = skin soaked gelatin solution NaOH and centrifuged and added ethanol, WNE = skin without soaked gelatin solution NaOH and added ethanol

weak water holding capacity which makes the water easily evaporate during drying.

The ash content was also not significantly different. The resulting gelatin was considered as good quality in term of ash content standard of BSNI [3] that 3.25%. This is in line with previous report. [4] recommended that ash content for a high quality gelatin was less than 0.5%. Low ash content of gelatin was affected by dissolved organic material during fat removal process.

Different treatments significantly (p <0.05) affected protein and fat content of the gelatin. The protein content was inversely related to fat levels. High level of protein in gelatin showed a good degree of purity.[14] stated that the gelatin was product of protein conversion produced by the hydrolysis of collagen, thus the protein was very high.

Low fat content was obtained in WNS treatment, but the result was not significantly different from WONS. [13] reported that lipids could be extracted at the same time as the collagen, and despite the mechanical removal and filtration step, it was possible that traces of lipids remained in the final product. Removal of fat by centrifugation is very effective because it can separate different molecular weights. [10] showed that the lower fat and ash contents represented efficient removal methods and showed a good gelatin quality. Furthermore, low fat level may maintain gelatin quality and extends its shelf life.

Table 2. Physical properties of chicken leg skin gelatin with different fat removal methods

| Variables | Treatments | | | | | | GMIA |
|---|---|---|---|---|---|---|---|
| | WONS | WONSE | WONE | WNS | WNSE | WNE | |
| pH | 4.00±0.52ab | 3.84±0.23b | 4.26±0.46a | 3.86±0.21b | 3.8±0.38b | 3.79±0.22b | 3,8-5,5 |
| viscosity (cP) | 12.43±0.64a | 7.70±0.46bc | 9.38±2.95b | 7.45±0.33bc | 6.83±0.33c | 8.63±0.30bc | 1.5-7 |
| Colour a | 1.92± 0.62 | 1.84±0.72 | 2.64±0.37 | 2.23±0.48 | 2.22±0.22 | 2.21±0.28 | |
| Colour b | 15.11±0.43b | 13.48±1.16c | 16.36±0.60a | 14.71±0.95b | 14.45±0.13bc | 15.50±0.44ab | |
| Colour L | 78.45±2.71 | 79.13±4.97 | 76.48±2.17 | 77.54±2.66 | 77.7±1.72 | 75.57±2.05 | |
| Yield (%) | 8.55±1.23 | 8.07±1.68 | 8.66 ±1.65 | 9.22±1.04 | 8.84± 0.57 | 9.92±0.53 | |

Different letters following the values in the same line indicate significantly different (P <0.05)

Notes: wons = skin without soaked NaOH and gelatin solution centrifuged, WONSE = skin without soaked NaOH and gelatin solution centrifuged and added ethanol, Wone = skin without soaked NaOH and gelatin solution was added ethanol, WNS = skin soaked NaOH and gelatin solution were centrifuged, WNSE = skin soaked gelatin solution NaOH and centrifuged and added ethanol, WNE = skin without soaked gelatin solution NaOH and added ethanol

The results exhibited that treatments significantly affected the pH ($p < 0.05$). Table 1 indicated that treatments without soaked with NaOH and centrifuged (WONS) was not different with all treatments soaked with NaOH and treatment with the addition of ethanol. Thus fat removal without NaOH and ethanol can be used. The pH value of the resulting gelatin is acid, since the acid is used in the conversion process of collagen into gelatin. [4] reported that the acid destabilizes the triple helical structure of collagen by disrupting acid labile cross-links at the telopeptide region and amide bonds of the triple helix as well as non-covalent intra and inter-molecular bonds. In this study, the concentrated acid (HCl 1.5%) was used, which was capable of penetrating into the skin tissue for pH reduction. The concentration of acid and pretreatment time influenced the physicochemical properties of the gelatin [7].

The gelatin viscosity is very important in its application to a product. Fat removal methods showed significant effect on the viscosity of the gelatin ($p < 0.05$). The highest viscosity was obtained from treatment of WONS (without soaked with NaOH and centrifuged). Fat decreasing by centrifugation possibly unaltered the molecular structure of gelatin that causes increasing viscosity. [15] stated that the viscosity was affected by the molecular weight and amino acid chain length of the gelatin.

Different methods of fat removal no significantly affected gelatin yield. Treatment of WNE (soaked with NaOH and addition of ethanol) resulted in the highest yield, but not different to other treatments, with the exception of the treatment WONSE (without soaked with NaOH and the solution was centrifuged and added by ethanol). The yield indicates the value of the quantity and effectiveness of a product processing. The quality and quantity of chicken leg skin gelatin shows inverse correlation. The treatment of the fat removal reduced the quantity of gelatin.

Table 2 exhibits color properties of gelatin including lightness (L*), red intensity (a), and yellow intensity (b). The differences in treatment significantly affected intensity of the yellow color ($p < 0.05$), but had no significant effect on the intensity of the brightness and the red color. The yellow color is influenced by raw material, extraction temperature, temperature and drying time, thus these factors contributed to non-enzymatic browning reaction of the product. [13] stated that maillard reaction occurred between the proteins and the traces of lipids during extraction process, which contribute to gelatin darkening.

## 4. Conclusion

Methods where skin samples have not been soaked in 0.05M NaOH and have been centrifuged were the best ones for producing quality gelatin from chicken legs *(Tarsometa tarsus)* skin.

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## Reference

[1] AOAC. *Official Methods of Analysis of The Association of Analitical Chemist*, Wahington . 1995

[2] Astawan M, Hariyadi P, Mulyani A. Analisis Sifat Reologi Gelatin dari Kulit Ikan Cucut. Jurnal Teknologi dan Industri Pangan. 2002.

[3] Badan Standar Nasional Indonesia. 06. 3735. Mutu dan Cara Uji Gelatin. Dewan Standarisasi Nasional, Jakarta. 1995.

[4] Benjakul, S., K. Oungbho, W. Visessanguan, Y. Thiansilakul, S. RoytrakulCharacteristics of gelatin from the skins of bigeye snapper, Priacanthus tayenus and Priacanthus macracanthus, Food Chem. 116 (2009) 445–451.2009.

[5] Brown E M, King G, Chen J M.. *Model of The Helical Portion of A Type I Collagen Microfibril*. Journal of American Leather Chemists Association, vol. 92, pp.1-7.1997

[6] Direktorat Jenderal Peternakan dan Kesehatan Hewan (Ditjennak Keswan). http://keswan.ditjennak.deptan.go.id. 2015..

[7] G. Boran, J. M. Regenstein. Optimization of gelatin extraction from silver carp skin, J. Food Sci. 74 E432–E441.2009.

[8] GMIA. *Standart Methods For The Testing of Edible Gelatin*. Offical Procedure of The Gelatin Manufacturers Institute of America, America.2012.

[9] Hunter lab. Hunter Lab Color Scale. Reston, VA. Hunter Associates Laboratories. USA.1996.

[10] Intidhar Bkhairia, Samiha Mhamdi, Mourad Jridi, and Moncef Nasri. New acidic proteases from Liza aurata viscera: characterization and application in gelatin production. International Journal of Biological Macromolecules. BIOMAC 6337.2016

[11] Liu, H.Y., J. Han dan S.D. Guo. Characteristics of the gelatin extracted from channel catfish (Ictalurus punctatus) head bones. Food Sci. Technol. 52:540 -544. 2009

[12] Puspawati *et al*. Isolasi Gelatin Dari Kulit Kaki Ayam Broiler Dan Karakterisasi Gugus Fungsinya Dengan Spektrofotometri Ftir. JURNAL KIMIA, vol. 79-87.2012

[13] Schrieber, R., & Gareis, H.Gelatin handbook - Theory and industrial practice. Wiley-VCH. 2007

[14] Sompie M , Mirah A D, Karisoh l. Pengaruh Perbedaan Suhu Ekstraksi Terhadap Karakteristik Gelatin Kulit Kaki Ayam. Biodiv Indon.Volume 1:792-795.2015.

[15] Stainsby. G..The gelatin and the sol-gel transformation. Dalam : Ward, A.G. dan Courts, A. (eds.). The Science and Technology of Gelatin, hal 109-165. Academic Press, New York. 1977

[16] Taufik M. 2011. Kajian Potensi Kulit Kaki Ayam Broiler sebagai Bahan Baku Gelatin dan Aplikasinya dalam Edible Film Antibakteri. [Disertasi]. Fakultas Peternakan Universitas Gadjah Mada, Yogyakarta.

# Physical and Chemical Characteristic of Chicken Meat from Kampung x Meat Type Crossbred Chicken

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## Abstract

The improvement of genetic quality of Kampung chicken as meat type production could be conducted through crossbreeding with broiler. Quality of meat is one of the parameters for the consumer to choosed meat. The aim of this study was to investigate physical and chemical characteristic of the kampung x meat type crossbred chicken. A total of 30 chicken 12 weeks aged were devided in five groups including: meat type chicken, Kampung chicken, F2 Kampung-meat typecrosbreed chicken faster growing, F2 Kampung-meat typecrossbreedchicken medium growing, F2 Kampung-meat typecrossbreed chicken slowergrowing. Chicken rearing in five groups were uniform. Samplesused were meat from breast (*Pectoralis superficialis*)muscle without skin. Each groups contained 3 heads of rooster and 3 heads of hent.Physical characteristic of meat were focused on pH, cooking loss, tenderness and water holding capacity. While the chemicals were water, fat and protein content. The data were analyzed using GLM (General Linear Model) to observe the effect of different groups associate with physical and chemical characteristic. The results showed that cooking loss, water holding capacity affected significantly ($P < 0.05$) in chicken groups, except the tenderness and pH.In case of chemical characteristic the protein content were found significanly higher in meat type. The present study demonstrated the differences in physical and chemical characteristic of meat existing between kampung x broiler crossbred chicken.

Keywords: meat quality, physical, chemical, crossbred chicken

## 1. Introduction

In Indonesia, the consumption of animal protein origin dominated by the meat type chicken. The average of meat type production in 2007 until 2015 to around 1.254.848 tons per year, while the production of kampung chicken is 283.471 tons per year, or about 18% [1]. Kampung chicken havebeen raised by most of the rural population of Indonesia and they represent an important source of meat. Kampungchicken are not able to provide consumption on daily basis because kampung chicken have low production. In addition, the local chicken nations also only contribute to the conservation of poultry genetic resources [2].

Contrast with the conditions, the broiler breeding industry in Indonesia was growing rapidly. However, it is still highly dependent on the supply of seeds and feed raw materials from abroad, are less able to keep people's food sovereignty Indonesia [3]. Dependence on imported raw materials can be pressed with local resources, one of which is the Kampung chicken. Production performance and quality of Kampung chicken meat can be increased by carried crosses with commercial broiler.

Quality of meat is one of the parameters for the consumer to choosed meat. According [4] the chemical composition has a close relationship with physical meat quality of the meat. It was argue further that the variation the largest meat component on the amount of fat. Fat meat has been recognized as the physical the meat quality varies component so much determined by fat content. In addition, the protein is the meat tissue arrangement has a very large role to change meat characteristics value.

The aim of this study was to investigate physical and chemical characteristic of the kampung x broiler crossbred chicken. A total of 30 chicken 12 weeks aged were devided in five groups including :meat type chicken, Kampung chicken, F2 Kampung-meat typecrosbreed chicken faster growing, F2 Kampung-meat typecrossbreedchicken medium growing, F2 Kampung-meat typecrossbreed chicken slowergrowing.

## 2. Material and Methods

### *2.1.Material*

A total of 30 chicken 12 weeks aged were devided in five groups including :meat type chicken, Kampung chicken , F2 Kampung-meat typecrosbreed chicken faster growing, F2 Kampung-meat typecrossbreedchicken medium growing, F2 Kampung-meat typecrossbreed chicken slowergrowing. Chicken rearing in five groups were uniform. Samplesused were meat from breast (*Pectoralissuperficialis*)muscle without skin. Each groups contained 3 heads of rooster and 3 heads of hent chickens.Physical characteristic of meat were focused on pH, cooking loss, tenderness and water holding capacity. While the chemicals were measured on water, fat and protein content.

### *2.2.Physical Analysis*

Physical analysis was done at the Laboratory of the Faculty of Ruminant Animal Husbandry, Bogor Agricultural University. Analysis of the physical quality of chicken used meat from breast (*Pectoralissuperficialis*). Physical characteristic of meat were focused on pH, cooking loss, tenderness and water holding capacity. Meat pH, measurement of pH value were followed [5] method by inserting a pH meter that has been calibrated into the meat, and then wait until the value showed on pH meter screen.

Tenderness, The degree of meat tenderness was indicated by the amount of force (kgcm-2) that required to cut the meat and indicated by the pointer tool Warner Bratzler device meat cutter which moves on a scale with the measurement sensitivity of 0.1 kg/cm2 [6].

Water Holding Capacity was ability of protein to hold the water in the meat. Value was measured by using planimeter with finding out the mount of water (mg) [7].

### *2.3.Chemical Analysis*

Water, fat and protein content in 5 groups of chicken was analysed according to the method described by AOAC [8] All content was expressed as persentase.

## 3. Result andDiscussion

Physical analysis was conducted to determine produced of chicken carcass quality. Analysis were conducted on four parameters such as pH, cooking loss, tenderness and water holding capacity. The results of physical analysis of chicken meat in 5 groups could be observed in Table 1 and Table 2. *pH value and tenderness* ,The results physical analysis showed that the mean pH value and tenderness did not significant among the five groups of chickens. But the result showed that the pH value in five group still in normal like the pH another livestock.

Table 1. Physical Characteristic of rooster in five groups

| Chicken type | pH | Cooking Loss (%) | Tenderness ($Kgcm^{-2}$) | Water holding Capacity (%) |
|---|---|---|---|---|
| Meat-Type | 5.51±0.06 | 36.41±3.44[a] | 2.35±0.31 | 28.32±1.51[c] |
| Kampong chicken | 5.52±0.09 | 29.62±4.00[b] | 2.11±0.28 | 31.46±1.05[a] |
| Kampong- meat type crossbreed faster growing | 5.40±0.1 | 37.26±2.34[a] | 2.41±0.65 | 29.11±0.45[bc] |
| Kampong- meat type crossbreed medium growing | 5.44±0.03 | 35.26±3.54[ab] | 2.36±0.09 | 30.74±0.96[ab] |
| Kampong- meat type crossbreed slower growing | 5.45±0.09 | 32.05±2.43[ab] | 2.03±0.20 | 29.91±0.57[abc] |

Note: Different superscriptsin the same column mean significant different ($p<0.05$).

Table 2. Physical Characteristic ofhent in five groups

| Chicken type | pH | Cooking Loss (%) | Tenderness ($Kgcm^{-2}$) | Water holding Capacity (%) |
|---|---|---|---|---|
| Meat-Type | 5.48±0.19 | 36.29±3.00 | 2.29±1.19 | 30.82±2.38 |
| Kampong chicken | 5.37±0.36 | 31.77±1.80 | 2.14±0.48 | 30.67±1.20 |
| Kampong- meat type crossbreed faster growing | 5.35±0.06 | 33.90±0.35 | 2.50±0.20 | 30.58±0.11 |
| Kampong- meat type crossbreed medium growing | 5.46±0.03 | 31.02±5.78 | 2.44±0.24 | 29.62±0.59 |
| Kampong- meat type crossbreed slower growing | 5.39±0.12 | 35.50±0.54 | 2.49±0.42 | 30.18±1.93 |

Note: Different superscripts in the same column mean significant different ($p<0.05$)

The mean pH value highest in Kampung chicken meat (5.52±0.09) and the mean pH value was lowest for the meat Kampong-crossbreed type of fast growth (5.40±0.11). Janisch et al. [9]reported a pH value in broiler chicken breast with three different strains ranged from 5.91 to 5.93. Chicken meat the village has a pH value of 5.10 to 5.40 [10].This difference allegedly as a result of crossbreeding and genetic differences in chicken and broiler. Stress before cutting, species, individual animals and the type of muscle, which affect glycolysis are factors that can produce variations in pH meat.

*Cooking Loss,* the results physical analysis showed that the mean cooking loss value of meat rooster has been significant among the five groups of chickens, where the mean cooking loss value highest in Kampung- meat type crossbreed fast growth (37.26±2.34) and the mean cooking loss value was lowest for the meat Kampong chicken (29.62±4.00). while the mean cooking loss values in the hens did not occur significantly from the chickens to the five groups. According Dilaga and Soeparno[11] a good quality meat has shrunk cook low due to loss of nutrients during cooking would be less. Reduction cooking shrinkage in food after boiling caused by reduced or loss of water content in food due to heating. The greater the heat given and the longer the heating will result in reduced water content in foodstuffs in large quantities. The use of heat in the cooking process is very influential on the nutritional value of foodstuffs [12].

Table 3. Chemical Characteristic of rooster chicken in five groups

| Chicken type | Water (%) | Fat (%) | Protein (%) |
|---|---|---|---|
| Meat-Type | 72.33±0.84$^{ab}$ | 0.21±0.08 | 25.26±0.42$^{a}$ |
| Kampong chicken | 71.87±0.49$^{ab}$ | 0.14±0.03 | 23.81±1.23$^{ab}$ |
| Kampong- meat type crossbreed faster growing | 71.28±0.82$^{b}$ | 0.19±0.09 | 24.08±0.75$^{ab}$ |
| Kampong- meat type crossbreed medium growing | 72.35±0.87$^{ab}$ | 0.24±0.22 | 22.13±1.48$^{b}$ |
| Kampong- meat type crossbreed slower growing | 73.38±1.40$^{a}$ | 0.27±0.04 | 22.89±0.95$^{b}$ |

Table 4. Chemical Characteristic ofhent chicken in five groups

| Chicken type | Water (%) | Fat (%) | Protein (%) |
|---|---|---|---|
| Meat-Type | 72.06±0.80 | 0.15±0.05 | 25.93±0.72$^{a}$ |
| Kampong chicken | 71.77±0.57 | 0.37±0.185 | 22.99±0.546$^{bc}$ |
| Kampong- meat type crossbreed faster growing | 71.56±0.64 | 0.35±0.307 | 24.12±1.04$^{b}$ |
| Kampong- meat type crossbreed medium growing | 71.70±1.52 | 0.22±0.187 | 23.57±0.40$^{bc}$ |
| Kampong- meat type crossbreed slower growing | 72.08±1.02 | 0.46±0.118 | 22.37±0.93$^{c}$ |

*Water holding Capacity*, the results physical analysis showed that the mean Water holding Capacity value of meat rooster has been significant among the five groups of chickens, where the mean Water holding Capacity value highest in meat Kampong chicken (31.46±1.05) and the mean Water holding Capacity value was lowest for the Meat-Type chickens (28.32±1.51). while the mean Water holding Capacity values in the hens did not occur significantly from the chickens to the five groups. Water holding capacity is the ability of meat proteins bind or hold the water content of the application as a response to external forces such as cutting, cooking, and grinding the meat [13]. The meat quality can be determined by the size of the water holding capacity, both technical and economical, both for industry or consumers directly as one important component in the storage of meat. High value of water holding capacity was mean that the cooking loss was not too high. Value HWC have negative correlated with the amount of free water that comes out.

*Water and fat content*, the results chemical analysis showed that the mean fat value of meat roosters and hens did not occur significantly among the five groups of chickens. The mean water value highest in Kampung- meat type crossbreed low growth (73.38±1.40) and the mean water value was lowest for the Kampung- meat type crossbreed fast growth (71.28±0.82).

The results chemical analysis showed that the mean protein value of meat roosters and hens has been significant among the five groups of chickens, where the mean protein value of the highest rooster contained in Meat-Type chickens (25.26±0.42) and the mean protein value was lowest for the Kampong- meat type crossbreed medium growth (22.13±1.48). while the hens mean value of the highest protein contained in

Meat-Type chickens (25.93±0.72) and the mean protein value was lowest for the Kampong- meat type crossbreed medium growth (22.37±0.93).

## Conclusion

The conclusion of this research that cooking loss, water holding capacity affected significantly (P <0.05) in chicken groups, except the tenderness and pH. In case of chemical characteristic the protein content were found significanly higher in meat type.

## References

[1] Direktorat Jendral Peternakan dan Kesehatan Hewan.2015. Statistik Peternakan. Direktorat Jendral Peternakan dan Kesehatan Hewan. Kementerian Pertanian, RI

[2] Sheng Z, ME Pettersson, X Hu, C Luo, H Qu, D Shu, X shen O Carlborg, N Li. 2013. Genetic dissection of growth traits in a Chinese indigenous x commercial broiler chicken cross. Genomics 14:151.

[3] Nataamijaya AG.2010. Pengembangan potensi ayam local untuk menunjang peningkatan kesejahteraan petani. J.LitbangPertanian. 29:4.

[4] Setiyono. 1987. Hubungan kualitas fisik dengan komposisi fisik dan kimia karkas daging domba local jantan yang diberi pakan dengan level energy dan berat potong berbeda. [tesis]. Yogyakarta (ID): PascasarjanaUniversitas Gajah Mada.

[5] Van Laack RLJM, Liu CH, Smith MO, Loveday HD. 2000. Characteristics of Pale, Soft, Exudative Broiler Breast Meat. *Poult. Sci*. 79: 1057-1061

[6] Suryati T, Arief II, Polii BN. 2008. Korelasi dan Kategori Keempukan Daging Berdasarkan Hasil Pengujian Menggunakan Alat dan Panelis. Anim. Prod. 10 : 88-193.

[7] Soeparno. 2005. Ilmu dan Teknologi Daging. Yogyakarta (ID): Gadjah Mada University Press.

[8] AOAC. 1999. Official Methods of Analysis of the Association of Official Analytical Chemist. Association of Official Analytical Chemist. Washington, USA.

[9] Janisch S, Krischek C, Wicke M. 2011. Color Values And Other Meat Quality Characteristics Of Breast Muscles Collected From 3 Broiler Genetic Lines Slaughtered At 2 Ages. Poultry Science. 90:1774–1781. doi:10.3382/ps.2010-01073.

[10] Dewi SHC. 2013. Kualitas kimia daging ayam kampong dengan ransum berbasis konsentrat broiler. Jurnal Agri Sains. Vol 4 No 6.

[11] Dilaga IWS, Soeparno. 2007. Pengaruh pemberian berbagai level clenbuterol terhadap kualitas daging babi jantan grower. BuletinPeternakan Vol. 31(4):200-208.

[12] Sundari D, Almasyhuri, Lamid A. 2015. Pengaruh Proses Pemasakan Terhadap Sumber Komposisi Zat Gizi 2011. Bahan Pangan Sumber Protein. Media Litbangkes. Vol 25 No 4 235-242.

[13] Soeparno. 2005. Ilmu nutrisi dan teknologi daging. Yogyakarta [ID] : Gadjah Mada University.

# Evaluated the Effect of Fermented Palm Sludge on Burgo Chicken Performance

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## Abstract

Bengkulu has a potential source of feed material as well as its availability is met throughout the year, and has been tested to animals is the oil sludge. Oil sludge can be obtained with the cheap price and the protein content is high enough. This research was aimed to evaluate the effect of fermented palm sludge (FPS) with *Neurosporasp* on burgo chicken performance. It was doing on 21 Desember 2014 until 15 February 2015 in Pagar Dewa, Kecamatan Selebar, Kota Bengkulu. This used 16 burgo chickens age 8-9 month that groups in 4 treatments with 4 samples that placed in individual cage. Treatments were P0 (diet control), P1 (diet with 5% FPS), P2 (diet with 10% FPS) and P3 (diet with15% FPS). Result showed that FPS did not improve ration consumption, did not reduce egg production percentation (%), did not reduce egg production (grain), did not reduce egg massa production (gram) and did not improve ration convertion($P>0.05$). The conclusion is fermented palm sludge until 15% in diet do not improve ration consumption, did not reduce egg production percentation (%), do not reduce egg production (grain), do not reduce egg massa production (gram) and do not improve ration convertion of burgo chicken. So, fermented palm sludge can use as diet until 15% and do not reduce performance of burgo chicken

Keywords: Fermented palm sludge, Burgo chicken

## 1. Introduction

Eggs are farm products that contribute to the achievement of adequate nutrition in the community. An egg obtained adequate nutrition perfect because eggs contain nutrients that are very good and easily digested by the body [1]. Lately symptoms of back to nature into something interesting. Upper middle class people who originally liked everything based technology is now starting to change to the situation that all natural. Demand for eggs and chicken eggs increased apparently participated affected by the phenomenon. Public perception of the chicken is the original chicken reared traditionally and is not given feed containing chemicals. Potential local chicken, which can be developed in the area of Bengkulu is burgo chicken that has huge potential and has a characteristic that is different from the other chickens [2]. Hen burgo can lay 20-25 point range in the period [3]. Lapse of time laying the average of 10 days, it is much faster than domestic poultry in general, which has an interval of spawning an average of 18 days.

In other side, Bengkulu has a potential source of feed material as well as its availability is met throughout the year, and has been tested to animals is the oil sludge. Oil sludge is a waste generated from extortion

palm fruit that has been through various processes to produce crude palm oil (CPO) or commonly known as crude palm oil [4]. Oil sludge can be obtained with the cheap price and the protein content is high enough. Protein content in the sludge of oil that has been fermented using a mold *Neurosprora sp* increased from 13.57% to 23.45 [5]. The granting of oil sludge fermentation (LSF) of 15% does not give negative effects to birds, especially for the performance of domestic poultry [6]. Expected use of oil sludge that has been fermented with *Neurosprora sp* could be expected to improve the performance of chicken burgo.

This study aimed to evaluate the effect of oil sludge that has been fermented with *Neurosprora sp* against burgo chicken performance.

## 2. Material and Methods

It was doing on 21 Desember 2014 until 15 February 2015 in Pagar Dewa, Kecamatan Selebar, Kota Bengkulu. This used 16 burgo chickens age 8-9 month that groups in 4 treatments with 4 samples that placed in individual cage. Treatments were P0 (diet control), P1 (diet with 5% FPS), P2 (diet with 10% FPS) dan P3 (diet with15% FPS), and used analysis of Completely Randomized Design (RCD). When a result is significant, it continue to Ducan's Mutliple Range Test (DMRT) to analysis means test.

## 3. Result and Discussion

### *3.1 Feed Consumption*

Based on the research results feed intake during the study had no significant (P>0.05). The use of oil sludge fermentation at the level of 15% in hen burgo not increase feed intake. It is the same with research (Sari et al., 2012), the use of LSF as much as 15% in laying chicken does not increase feed intake compared with control treatment.

Chicken consume rations is none other than to meet the basic needs of life, growth and reproduction Feed intake is strongly influenced by the environment and the balance of nutrients, the quality of rations, breeds, rate of growth, body weight and level of production. In selecting a feed, the chicken will use his instincts to choose the feed that is, if the feed contains enough nutrients then the level of palatability of the chicken was high on the contrary, if the feed is less containing nutrients required for the necessities of life, the chickens will continue to consume feed that meets every aspect of their daily needs. Increasing and decreasing feed intake is influenced by energy content, of this study on the energy content of the ration so that the same relative feed consumption showed no significant.

Average consumption of hen burgo rations grams/head/day during the study are shown in Table 1.

### *3.2 Percentage of Egg Production*

The influence of the use of FPS against the percentage of egg production during the study showed no significant (P>0.05), this can be seen in Table 1. The use of oil sludge fermentation to the extent of 15% did not increase egg production, but also does not reduce the percentage of egg production in burgo chicken. The use of sludge oil to the extent of 15% in laying chicken showed no negative symptoms [4]. The use of FPS is not optimal to increase egg production due to FPS deficiency in amino acids lysine and mentionin [5].

Although the treatment effect was not significant (P>0.05) on the percentage of the average production during the study, but it can be seen that the percentage of egg production related to feed intake (Table. 1). In each treatment P0: 49.56 g/head/week (42.86), P1: 43.52 g/head/week (23.21), P3: 42.28 g/head/week (28.57). The number of low feed intake can cause a drop in egg production and feed intake otherwise high can increase the amount of egg production [9].

Table1. The result in this research

| Variables | Treatments | | | |
|---|---|---|---|---|
| | P0 | P1 | P2 | P3 |
| Feed Consumption (g/head/day) | 53.46±4.34 | 53.62±9.19 | 55.46±6.32 | 60.91±12.82 |
| Percentage of Egg Production (%) | 51.34±9.82 | 45.09±16.00 | 41.07±10.00 | 51.34±16.58 |
| Egg Production (grain/head) | 28.75±5.50 | 25.25±8.96 | 23.00±5.60 | 28.75±9.29 |
| Production of Egg Mass (g/head) | 765.96±134.49 | 734.08±288.78 | 648.01±181.72 | 810.90±264.79 |
| Feed Convertion | 3.98±0.61 | 4.47±1.37 | 4.96±0.84 | 4.41±0.92 |

*3.3 Egg Production (Grain)*

Results of analysis of variance showed that the use of FPS against egg production (grain) during this research not significant (P> 0.05). According to [10] ration quality and environmental conditions greatly affect the number of eggs produced. Rations with low protein content are not capable of supporting high egg production. The protein content of the ration in this study are relatively the same, so do not reduce the production of eggs. In this research use of oil sludge fermentation at 5%, 10%, and 15% do not reduce the production of eggs.

Maintenance aspects affect performance both in terms of consumption, production and reproduction. The chickens are kept intensively necessities of life governed by the breeder, unlike chickens are reared extensively dependent to ambient conditions both feed and place of residence. In this study burgo chickens can produce 23-28 eggs per period, it agrees with [11] which suggests that the maintenance of burgo intensive chicken can increase the amount of egg production per period.

*3.4 Production of Egg Mass*

Results of analysis of variance totaling mass production of chicken eggs burgo do not affect the provision of FPS to the extent of 15% during the study (Table 1). An important factor influencing the size of the egg is protein and amino acids, about 50% of dry matter so that eggs contain protein provides amino acids in protein synthesis is very necessary for the production [12]. According to [13], linoleic acid content contained in the ration able to sustain the weight of eggs produced by laying hens. The balance of amino acids and linoleic acid contained in the study, is quite balanced rations that do not cause a decrease in egg mass production.

*3.5 Feed Convertion*

Feed conversion was calculated to add the feed consumed divided by the total weight of eggs produced. Based on the study results during burgo chicken feed conversion in treatment P0, P1, P2 and P3 during the study showed no significant (P>0.05). [14] explains that the feed conversion rate is the number of ration consumed a chicken in a certain time to form a meat or eggs. Factors affecting feed conversion rate among other strain, feed quality, state of the cage and gender. Based on this study feed quality is relatively the same, so do not improve feed conversion and also does not reduce the value of feed conversion [12]. Use of FPS to the extent of 15% does not improve the feed conversion but the use of LSF to some 15% also does not reduce the value of feed conversion, so the use of LSF in burgo chicken feed could be used to the extent of 15%.

**Conclusion**

It can be concluded that the use of oil sludge fermentation in the ration to the extent of 15% does not decrease feed consumption, egg production and feed conversion burgo chicken. Utilization of oil sludge fermentation can be used as chicken feed burgo to the extent of 15% without lowering the production performance of burgo chicken.

## References


[1] Sudaryani T. 2003. *KualitasTelur*. Cetakan Keempat. PT Penebar Swadaya (ID): Jakarta

[2] Putranto. H. D. 2011. Pengaruh Suplementasi Daun Katuk terhadap Ukuran Ovarium dan Oviduk serta Tampilan Produksi Telur Ayam Burgo.*Jurnal Sains Peternakan Indonesia*.2(6): 103-114.

[3] Setianto J. 2009. *Ayam Burgo Ayam Buras Bengkulu*. IPB Press. Bogor.

[4] Sinurat AP. 2003. Pemanfaat Lumpur Sawit untuk Bahan Pakan Unggas.*Wartazoa*. 13 (2): 39-47.

[5] Fenita Y, Santoso U,. Prakoso H. 2010. Pengaruh Lumpur Sawit Fermentasi dengan *Neurospora sp* terhadap Performans Produksi dan Kualitas Telur. *Jurnal Ilmu Ternak dan Veteriner*. 2 (15):88-96.

[6] Sinurat AP, Purwadaria, Pasaribu T, Darma J, Bintang IAK,Torgatop MH. 2001. Pemanfaatan Lumpur Sawit Fermentasi Untuk Ransum Unggas. Penggunaan Produk lumpur Sebelum dan Sesudah Dikeringkan Dalam Ransum Ayam Kampung Yang Sedang Tumbuh. *Jurnal Ilmu Ternak Veteriner*.

[7] Sari R.P, D. Kaharuddin,U. Santoso, Y.Fenita . 2012.Pengaruh Suplementasi Ekstrak Daun Katuk (*Sauropusandrogynus*) dalam Ransum Berbasis Lumpur Sawit Fermentasi terhadap Performans Ayam Ras Petelur. *Jurnal Sains Peternakan Indonesia*. 2 (7):81-92.

[9] Amrullah IK. 2003 *Nutrisi Ayam Petelur*. LembagaGunung Budi (ID): Bogor.

[10] Prasetyo H andKetaren P. 2005. Interaksi antar bangsa itik dan kualitas ransum pada produksi dan kualitas telur itik lokal.Seminar Nasional Teknologi Peternakan dan Veteriner Vol 3 (4) : 230-235. Balai Penelitian Ternak (ID): Bogor.

[11] Warnoto. 2002. Transformasi Genetik Ayam Burgo Dalam Rangka Penyediaan Bibit dan Peningkatan Produksi Telur Ayam Kampung di Bengkulu. *Jurnal Pengembangan dan Penerapan Teknologi*.

[12] Anggorodi. 1994. *Ilmu Makanan Ternak Umum*. Gramedia (ID): Jakarta.

[13] Montesqrit. 2007. Penggunaan bahan pakan sebagai penyalut dalam mikroenkapsulasi minyak ikan lemuru dan pemanfaatannya dalam ransum ayam petelur [Disertasi].Sekolah Pasca Sarjana. Institut Pertanian Bogor: Bogor.

[14] Rasyaf M. 1994. *Makanan ayam Broiler*. Kanisius (ID): Yogyakarta.

# SCFA Profile of Rice RS Fermentation by Colonic Microbiota, *Clostridium butyricum* BCC B2571, or *Eubacterium rectale* DSM 17629

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## Abstract

Resistant starch type 3 (RS3) produced from various high amylose food sources through retrogradation or enzymatic process is known to have physiological function as dietary fiber. Fermentation of RS3 by colonic microorganism produced SCFA (acetate, propionate, and butyrate), maintain the health of colon, balance of gut microbiota and prevent colon cancer. RS3 in this study was made from IR-42 and Inpari-16 broken rice by enzymatic treatment (combination of amylase-pullulanase). The Resistant Starch was fermented for 12 and 24 hours by colonic microbiota, *Clostiridium butyricum* BCC-B2571, or *Eubacterium rectale* DSM 17629. SCFA produced was analyzed by gas chromatography. The result showed that after enzymatic process, the RS3 content of IR-42 (41.13%) was not significantly different ($p<0.05$) from that of Inpari-16 (37.70%). Treatment by amylase-pullulanase combination was advantageous to increase their RS3 content. The results showed that high concentration of acetate (82.5 mM) was produced by colonic microbiota after 12 hours fermentation. The best propionate content (7.5 mM) was also produced by colonic microbiota after 12 hours fermentation and best concentration of butyrate (6.8 mM) was produced by colonic microbiota after 24 hours fermentation. It is clear that utilization of colonic microbiota rather than single colony was better in the production of SCFA.

Keywords: Resistant starch, colonic microbiota, *Clostridium butyricum* BCC-B2571, *Eubacterium rectale* DSM 17629, SCFA.

## 1. Introduction

Healthy digestive system is increasingly important, in line with changes in diet and lifestyles. Imbalanced diet, such as not enough consuming dietary fiber, can harm health of the colon that can lead to colon cancer. Cancer cases in 2012 covered approximately 1.4 millionin the world, or third after lung and breast cancer [1]. There are about 80% of CRC cases related to diet, 15% of which are caused by genetic while the rest comes from other factors, including environment [2]. This shows that in fact CRC can be avoided, and food with high dietary fiber such as Resistant Starch type 3 (RS3) can be of use for cancer prevention.

Resistant starch (RS) refers to starch and starch degradation products that escape from digestion in the small intestine of healthy individuals. Resistant starch, not digested in the small intestine, has physiological function as dietary fibers, beneficial to prevent colon cancer.Some types of resistant starch (RS1, RS2, and RS3) are fermented by the colonic microbiota and produce metabolite such as short chain fatty

acids (SCFA):acetate, propionate, butyrate, and lactate. The metabolites involved in many factors related to the health of the colon, including the composition of gut microbiota, regulation of the immune system, inhibition proliferation of pathogens, intestinal motility, and energy recovery. Natural fermentation is carried by some microbes include the genus *Eubacterium*, *Peptostreptococci*, *Clostridia*, *Roseburia* spp, and *Butyrofibrio fibrisolven*[3].

Applications of resistant starch in food products as prebiotics and food ingredients are expected to maintain the health of colon, balance of gut microbiota and prevent colon cancer. Various studies had been conducted to produce RS flour. RS3 can be produced from high starch materials such as rice, sweet potato, banana, cassava. Rice is food source, largely composed of starch. Rice milling will produce broken rice. Currently, broken rice utilization is still limited, even into waste or animal feed. Broken rice's potential to be developed as RS, can be the solution to increase its the economic value. Broken rice in 2013 reached about 16% of MPD (milled rice), or about 11.4 million tons [4]. Purwani et al. [5] reported RS3 content of rice was higher in the combination of amylase-pullulanase by 27%, compared with the treatment of these two enzymes separately. Tan[6] reported RS3 content of rice increased to 49.7% with combination of amylase-pullulanase. Guraya et al.[7] reported RS3 content of rice by 13% by the method of hydrolysis by pullulanase. Kim et al. [8] reported RS3 of rice starch with α-amylase hydrolysis produced RS3 content approximately 16%.

Zhao and Lin [9] reported RS3, from corn starch was hydrolyzed with citric acid, improved to liquid infant's stools or healthy adults fermentation of RS3 at $37^0$C for 0, 12, 24 hours, produce increase molar of butyric acid increased in line with time fermentation. Sharp and Macfarlane [10] reported that RS could stimulate the growth (in vitro) of butyrate-producing bacteria of the genus Clostridia. Yang et al. [11] reported RS fermentation could affect colonic microbial composition and production of SCFA.

In our study, RS3 was made through the combination of retrogradation (interaction between amylose fractions) and enzymatic hydrolysis combination (amylase-pullulanase).Then, RS3 was fermented by colonic microbiota, *Clostridium butyricum* BCC-B2571 or *Eubacteriumrectale* DSM 17629 as a model bacterial strain.

The aim of this research was to produce RS3 from broken rice through the combination of retrogradation (interaction between amylose fractions) and enzymatic hydrolysis (amylase-pullulanase), and to analyze SCFA produced by fermentation of colonic microbiota, *Clostridium butyricum* BCC-B2571 and *Eubacteriumrectale* DSM in medium supplemented with rice RS3. This study knows the composition of SCFA during fermentation by colonic microbiota, *Clostridium butyricum* BCC-B2571 and *Eubacteriumrectale* DSM in medium supplemented with RS3 of rice.

## 2. Material and Methods

### *2.1. Rice and Chemicals*

Broken rice IR-42 and Inpari-16 were obtained from the Indonesia Center for Rice Research, Sukamandi, Indonesia. Two types of starch degradation enzymes were from Novozymes. Enzymeused were: alpha-amilase (Liquozymes® Supra) 135 KNU/g and Pullulanase (Dextrozymes® DX 1.5X) 510 NPUN/g.

### *2.2.Bacterial strain and culture media*

Colony microbiota was derived from feces of healthy adult subject, 30-50 years, who did not take antibiotics for at least 3 months and had no history of gastrointestinal disease. Pure culture of *C. butyricum* BCC-B2571 was obtained from Culture Collection of Indonesia Research Center for Veterinary Sciences (IVETRI), Indonesia. *Eubacterium*

*rectale* DSM 17629 was obtained from DSMZ, Germany.The basal medium for colonic microbiota and *C. butyricum*BCC-B2571 consisted of (g/L):yeast extract 3, beef powder 10, peptone 10, glucose 5, soluble starch 1, NaCl 5, Na- acetate 3 and cysteine hydrochloride 0.5.The pH was adjusted to 6.8 The basal medium of *Eubacterium rectale* DSM 17629 consisted of (g/L) : tryptone 5, bacteriological peptone 5, yeast extract 10, beef extract 5, glucose 5, Tween 80 1 mL, resazurin 0.001, $CaCl_2$ 0.01, $MgSO_4$ 0.02, $K_2HPO_4$ 0.04, $KH_2PO_4$ 0.04, $NaHCO_3$ 0.4, NaCl 0.08, Vitamin K1 0.0002. The pH wasadjusted to 7.0.

*2.3. Production of resistant starch*

Rice was extracted by an alkaline solution [12]. Rice flour (500 g) was mixed with 0.045 M NaOH 1L, stirred constantly for 1 hour, filtered with 2 layers by filter cloth. The filtrate was collected, centrifuged at 1500 g, 4°C for 7 min. The supernatant was discarded, the upper sediment (protein) was separated from the bottom sediment (starch). Starch fraction was mixed with 0.045 M 1 L NaOH centrifuged,starch fraction was suspended in $H_2O$ 250 mL, andwas neutralized with 1 M HCl, followed by twice. Starch was dried in 40°C oven for approximately 18 hours.Milled rice starch was stored at 4°C until use.

Rice starch was processed into RS3 following Kim et al. [13] with modifications. Starch (50g) was suspended in 200 mL $H_2O$, boiled (100°C, 10 min), removed to room temperature. The gel was vacuum sealed in a retort pouch and autoclaved at121°C, 15 psi for 1 h, and stored at 4°C for 12-14 h, to induce retrogradation.Retrograded starch was suspended in 1 L of $H_2O$and blended high speed for 2 min. The starch suspensionwas enzymatic hydrolyzed, by 1 mL α-amilase for 3 h at 85°C, continued with 1 mL of pullulanase for3 h at 55°C. The hydrolyzed starchwas centrifuged (1500 g). The residuewas collected and stored at 4°C for 16-18 hours, suspended in$H_2O$ 250 mL and homogenized for 2 min by homogenizer. The suspension dried with a spray dryer, with inlet temperature 160°C.

*2.4. Analysis of rice starch*

Moisture, ash, protein and minerals in were analyzed [14], whereas the amylose content of starch were analyzed using colorimetric methods [15].

*2.5.Determination of resistant starch*

RS3 content was analyzed according to by Goni et al. [16].RS3 50 mg was dispersed in 5 ml KCl-HCl pH 1.5 and incubated with 4400 units of pepsin solution at 40°C in shaker incubator for 1 hour to remove the proteins.Trismaleate buffer 0.1 M pH 6.9 (4.5 mL) was added and incubated with amylase (100 units) for 16 hours at 37°C to hydrolyze the digestible starch. Sample was then centrifuged (1000g, 15 min) twice. The supernatant was discarded while the residu was moistened with 1.5 mL $H_2O$ and dissolvedwith 1.5 mLKOH4M. RS solution was mixed with HCl 2 M and Na-acetate buffer 0.4M pH 4.75, then incubated with 100 units amyloglucosidase at 55°C for 45 minutes. The suspension was centrifuged (1000g for 15 minutes) and the supernatant was collected. Glucose in the supernatant was measured by the phenol-sulfuric acid method [17]. RS was calculated as glucose x 0.9 and expressed as percent of RS in sample.

*2.6.In Vitro Fermentation*

Growth medium 20 mL, with varioustype of RS3 (2%), was distributed in the serum bottles flushed with $CO_2$, sealed with a butyl rubber septum and sterilized at 121°C 15 min. It was inoculated with 1 mL of 24 h pre-culturedbacterial strain (at about $10^9$ CFU/mL), and incubated under anaerobic condition at 37°C in water bath.

Fermentation was carried out for 12 and 24 h, (three replications). In another in vitro fermentation, glucose was used as the only

carbon source (concentration 2%), run as control.

*2.7. Gas production and pH measurement.*

Gas production (mL) was measured by channelingthe gas in the serum bottle to expand into glass syringe. The pH of the cultures was determined by pH meter.

*2.8.Analysis Short chain fatty acid*

Media fermentation was centrifuged (3000g, 10 minutes). The supernatant was filtered with a membrane (0.45µm) and stored at 4°C until use. Samples (1 mL) was injected into gas chromatography instrument (Agilent Technologist, 7890A GC System) equipped with a flame ionization detector (FID) and HP Innowax 19091-136 coloum column (60 m x 0.250 mm). The carrier gas was$H_2$ at speed 1.8 ml/min. The oven temperature was maintained at 90°C for 0.5 min, and then increased to 110°C at a rate of 10°C/min, increased to 170°C at a rate of 5°C/min and finally increased to 210°C at a rate of 20°C. Injector and detector temperatures were 275°C. SCFA mixture containing acetate, propionate and butyrate at specific concentration were used as standard

*2.9.Statistical analysis*

All data were expressed as means ± SE from at least three independent trials. Differences between the mean values ofmultiple groups were analyzed by one-way analysis of variance (ANOVA), Duncan test was carried out to compare the data between treatments, independent t-test, and Pearson correlation coefficients, $p< 0.05$ was considered a significant different. SPSS 22 software was applied to analyze the data.

## 3. Result and Discussion

*3.1.Chemical composition of rice starch*

Chemical composition of native rice starch is shown in Table 1.

Table 1. Extracted rice starch composition

| Chemical component | IR-42 | Inpari-16 |
|---|---|---|
| Amylose (%) | 34.09±0.17 | 28.28±0.13 |
| Moisture (%) | 8.28±0.15 | 6.99±0.25 |
| Ash(%) | 0.15±0.05 | 0.09±0.001 |
| Crude Fat (%) | 0.29±0.04 | 0.26±0.02 |

*3.2.Resistant starch content*

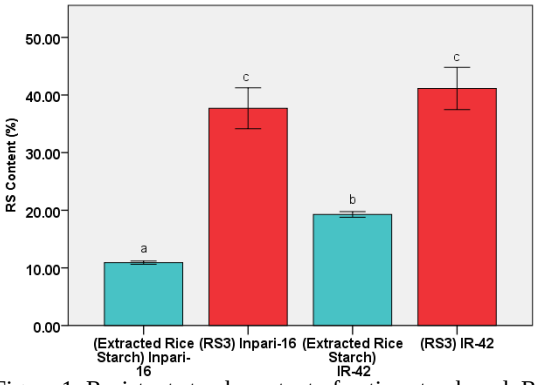


Figure 1. Resistant starch content of native starch and RS3 of rice.

Amylose content of rice starch was 34.09% (IR-42) and 28.28% (Inpari-16) (Table 1). The rice amylose of IR-42 and Inpari 16 were 26.70% [18] and 22.7% [19]. IR-42 was classified as high amylose rice and Inpari-16 as intermediate amylose rice. Chemical composition of IR 42 differed from Inpari 16, depended on the variety of rice. The high rice starch content, deserved to be further processing into RS3.

The rice starch should be gelatinized and retrograded before hydrolyzed by enzymes. Gelatinization changed granular structure so that the starch became more accessible to enzymes. The storage at 4°C induced retrogradation, crystallization and formation of the starch matrix which had undergone gelatinization. Alpha-amylase hydrolyzes (1,4)-α-D-glycosidic of the rice starch and produces oligosaccharides, maltose and glucose. Pullulanase hydrolyzes (1,6)-α-D-glycosidic of the amylopectin and produces linear oligosaccharides, maltose and glucose.

The RS content of native IR-42 was higher than that of Inpari-16 (Fig. 3). Amylase-pullulanase treatment increased the

RS content : IR-42 (41.13%) and Inpari-16 (37.70%). The amylose content starch had significant contribution to RS formation. Interaction between amylose chains could form double helical structure, stabilized by hydrogen bonds and more resistant to amylase. Higher amylose content could produce RS3 with high RS content. Amylopectin hydrolyzedby pullulanase produced linear oligosaccharides andexpected to increase the double helical structure. The results obtained resistant starch content was higher than that obatained earlier by Purwani et al. [5].

### *3.3. Effect of resistant starch on pH and gas during in vitro fermentation*

The effect of resistant starch on pH during in vitro after 12 and 24 hours fermentation are shown in Figure 2. The pH values decreased in all treatment, compared with initial medium, after 12 or 24 hours each microbe. However, pH after 12 and 24 hours did not show significant change ($p<0.05$). Purwani et al. [5] reported that fermentation of rice resistant starch (1%) treated by amylase and pullulanase, resultedin pH 4.5 formed by *C.butyricum* BCC-B2571 or *E.rectale* DSM 17629 after 48 hours fermentation.

The effect of resistant starch on gas during in vitro after 12 and 24 hours fermentation is shown in Figure 3. Gas production was different to resistant starch after 12 and24 hours fermentation, except when*E. rectal*e 17629 was used. Purwani et al. [5] reported that rice resistant starch (1%), gas 8.70mL by *C.butyricum* BCC-B2571 and 10.60mL by *E.rectale*DSM 17629 after 48 hours fermentation

### *3.4. Production of short chain fatty acids during in vitro fermentation*

SCFA profile resulted from12 or 24 hours fermentation by different microbesis shown in Figure 4. The main product after 12 hours fermentation of RS3 on eachmicrobewas acetate (18.68 to 82.47 mM), propionate (1.95 to 7.45 mM) and butyrate (0.89 to 6.78 mM). Molar (mM) of acetate:propionate: butyrate after 12 hours fermentation by colonic microbe were 82.47:7.45: 6.44in medium supplemented with RS3 IR-42 and 32.04:2.45:0.89 inmedium supplemented with RS3 Inpari-16. Molar (mM) of acetate:propionate: butyrate after 12 hours fermentation by *C.butyricum* BCC-B2571 were 74.93:6.10: 6.78 in medium supplemented with RS3 IR-42 and 19.18:1.95: 2.51 in medium supplemented with RS3 Inpari-16. Molar (mM) of acetate:propionate: butyrate after 12 hours fermentation by *E. rectale* DSM 17629, were 21.62:5.33: 5.37 in medium supplemented with RS3 IR-42 and 18.68:5.67:5.65 in medium supplemented with RS3 Inpari-16. Total SCFA formed by each microbe, in medium supplemented with RS3 IR-42 was higher than that with RS3 Inpari-16,on acetate, propionate and butyrate, and when compared with the control, total SCFA in medium supplemented with RS3 Inpari-16 on for each microbe was lower compared to control (glucose).

The main product after 24 hours fermentation of RS3 on each microbe was acetate (18.09 to 63.28 mM), followed by butyrate (4.80 to 6.84 mM) and propionate (3.45 to 6.27 mM). Molar (mM) of acetate:propionate: butyric after 24 hours fermentation by colonic microbiota were 63.28:6.27: 6.84 in medium supplemented with RS3 IR-42 and 48.64:3.45: 4.86 in medium supplemented withRS3 Inpari-16. Molar (mM) of acetate:propionate: butyrate after 24 hours fermentation by*C.butyricum* BCC-B2571 were 59.45:4.53: 6.39inmedium supplemented with RS3 IR-42 and 35.06:3.98: 4.80in medium supplemented with RS3 Inpari-16.

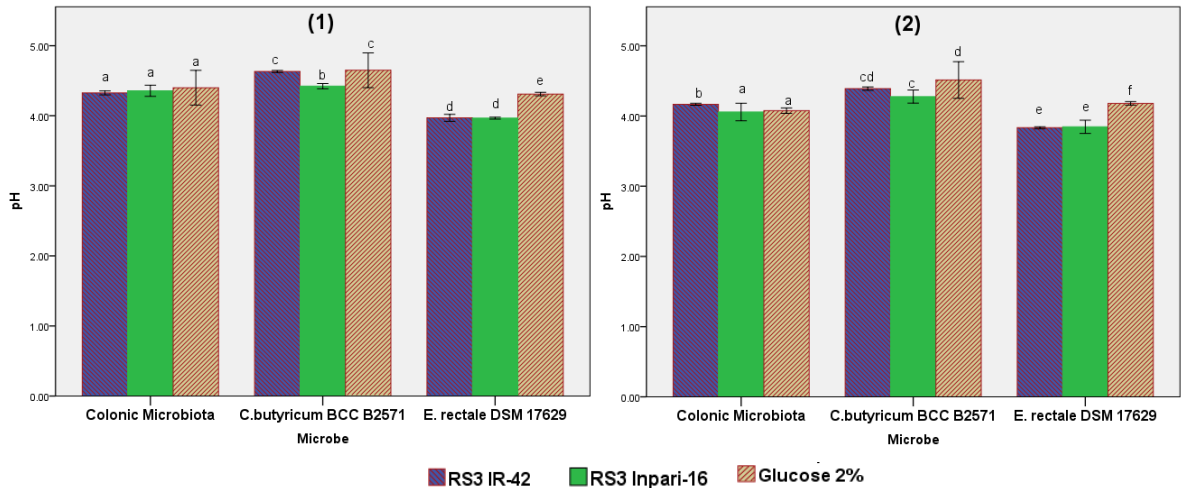


Figure 2. Profile of pH during fermentation by colonic microbiota, *C.butyricum* BCC-B2571 or *E. rectale* DSM 17629 in different media after (1) 12 and (2) 24 hours fermentation. Mean values above bar followed by the different letters represent significant different ($p<0.05$)

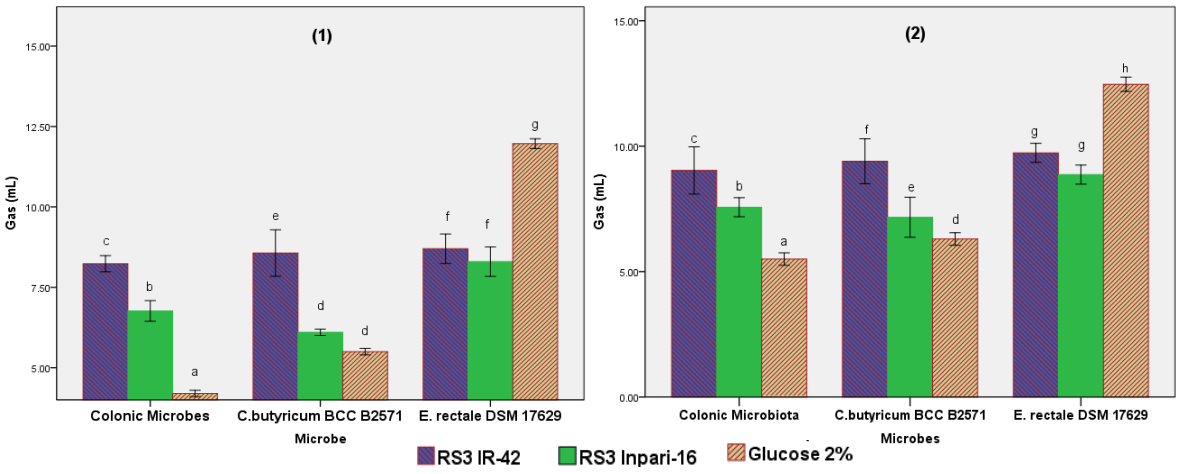


Figure 3. Profile of gas during fermentation by colonic microbiota, *C.butyricum* BCC-B2571 or *E. rectale* DSM 17629 in different media after (1) 12 and (2) 24 hours fermentation. Mean values above bar followed by the different letters represent significant different ($p<0.05$)

Molar (mM) of acetate:propionate: butyrate after 24 hours fermentation by*E. rectale* DSM 17629 were 28.27:5.74: 6.48 in medium supplemented with RS3 IR-42 and 18.09:4.97: 3.59 in medium supplemented with RS3 Inpari-16. Total SCFA formed by each microbe, in medium supplemented with RS3 IR-42 produced SCFA higher than in medium supplemented withRS3 Inpari-16 well on acetate, propionate and butyrate.

Our studyshowed that after 12 hours fermentation, *C.butyricum* BCC B2571produced butyrate higher ($p<0.05$) in medium supplemented with RS3 IR42 (6.78 mM) than in medium supplemented with RS3 Inpari-16 (2.51 mM).Meanwhile after 24 hours fermentation, the colonic microbe produced butyrate higher ($p<0.05$) ) in medium supplemented with RS3 IR42 (6.84 mM) than in medium supplemented with RS3 Inpari-16 (4.86 mM).

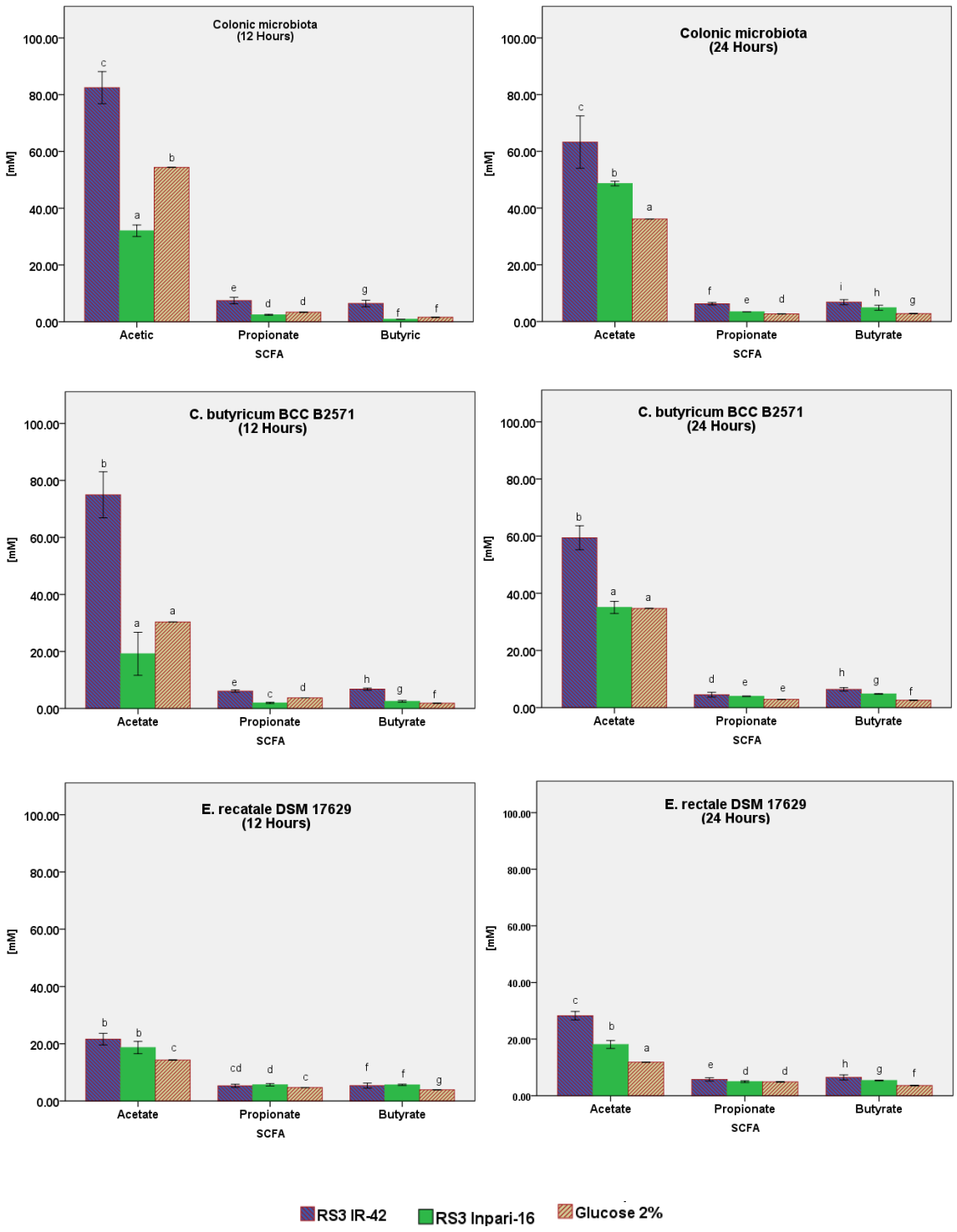


Figure 4. SCFA Profile by Colonic microbiota, *C.butyricum* BCC B2571, and*E.rectale* DSM 17629 in different medium supplemented after 12 or 24 hours fermentation. Mean values above bar followed by the different letters represent significant different ($p<0.05$)

Table 2. SCFA Profile by different resistant starch after 12 and 24 hours fermentation[a].

| Resistant Strach | SCFA after fermentation | Colonic microbiota | *C.butyricum* BCC B2571 | *E. rectale* DSM 17629 |
|---|---|---|---|---|
| RS3 IR-42 | Acetate (mM) 12 h | 82.47 | 74.93 | 21.62 |
| | Acetate (mM) 24 h | 63.28 | 59.45 | 28.27 |
| | ***p*-value** | **0.037** | **0.042** | **0.010** |
| | Propionate (mM) 12 h | 6.27 | 6.10 | 5.33 |
| | Propionate (mM) 24 h | 7.45 | 4.53 | 5.74 |
| | ***p*-value** | | **0.043** | |
| | Butyrate (mM) 12 h | 6.44 | 6.68 | 5.37 |
| | Butyrate (mM) 24 h | 6.84 | 6.39 | 6.48 |
| | ***p*-value** | | | |
| RS3 Inpari-16 | Acetate (mM) 12 h | 32.04 | 19.18 | 18.68 |
| | Acetate (mM) 24 h | 48.64 | 35.06 | 18.09 |
| | ***p*-value** | **0.000** | **0.025** | |
| | Propionate (mM) 12 h | 2.45 | 1.95 | 5.67 |
| | Propionate (mM) 24 h | 3.45 | 3.98 | 4.97 |
| | ***p*-value** | **0.000** | **0.000** | |
| | Butyrate (mM) 12 h | 0.89 | 2.51 | 5.65 |
| | Butyrate (mM) 24 h | 4.86 | 4.80 | 5.38 |
| | ***p*-value** | **0.001** | **0.000** | |

[a]Only significant independent t-test reported ($p < 0.05$)

Table 3. Pearson correlation coefficients for RS in different microbes versus SCFA concentrations

| Microbe | 12 Hours | | | 24 Hours | | |
|---|---|---|---|---|---|---|
| | Acetate | Propionate | Butyrate | Acetate | Propionate | Butyrate |
| *Colonic microbiota* | 0.991[a] | 0.966[a] | 0.972 | | 0.986 | |
| *C.butyricum* BCC B2571 | 0.975 | 0.991 | 0.991 | 0.976 | | 0.912 |
| *E. rectale* DSM 17629 | | | | 0.975 | | |

[a] $p < 0.01$, Only significant correlations reported ($p < 0.05$)

Table 2 shows the SCFA profile after 12 and 24 hours fermentation of different RS3. In medium supplemented with RS3 IR-42, acetate production by colonic microbiota, *C.butyricum* BCC-B2571 or *E. rectale* DSM 17629 showed different molar. Molar of acetate produced by colonic microbe and*C.butyricum* BCC-B2571 was higher after 12 than 24 hours fermentation ($p < 0.05$). Meanwhile *E. rectale* DSM 17629 produced acetate more higher after 24 than 12 hours fermentation. RS3 IR-42 fermented by *C.butyricum* BCC-B2571 produced different propionate molar after 12 and 24 hours fermentation, while propionate produced higher after 12 hours fermentation. In medium supplemented with RS Inapri-16, acetate, propionate, and butyrate productions by colonic microbiota and*C.butyricum* BCC-B2571 showed different molar after 12 and 24 hours fermentation. RS3 Inpari-16 fermented by colonic microbiota and *C.butyricum* BCC B2571, after 24 hours fermentation produced acetate, propionate, and butyrate significantly higher than after 12 hours fermentation.

The study result showed, proportion and content of SCFA was dependent on bacteria strain and varieties of RS3. In our study, butyrate molar produced was higher than those produced by colonic microbiota in medium supplemented with RS3 HACS treated by amylase [20]. After 24 hours fermentation, acetate ranged from 14.67 to 17.14 mM, propionate ranged from 4.03 to 5.29mMand butyrate ranged from 0.14 to 0.75 mM. Our study showed that produced of SCFA by colonic microbiota was higher than SCFA by colonic microbiota in medium sup-

plemented with apple juice extracts [21].

High concentration of butyrate was produced after fermentation 48 hours of rice RS3 (treatment by amylase-pullulanse) by *C.butyricum* BCC B2571 and *E. rectale* DSM 17629, but low acetate by *C.butyricum* BCC B2571 than in our study [5]. Butyrate was produced by *C.butyricum* BCC B2571 and *E. rectale* DSM 17629 were respectively 27.05 mM and 22.46 mM, while acetate by *C.butyricum* BCC B2571 was 48.88 mM. Butyrate production by colonic microbe and *C.butyricum* BCC B2571 increased after 24 hours fermentation in medium supplemented with RS3 Inpari-16. In our study,accumulation of acetate molar indicated that the butyrate was produced by colonic microbiota, *C.butyricum* BCC B2571 and *E. rectale* DSM 17629 via butyryl-CoA transferase.Miller and Wolin [22] was reported the pathway of acetate, propionate, and butyrate synthesis by human colonic microbiota. At the final step of butyrate synthesis, there are two alternatives pathway, butyrate kinase pathway and a butyryl-CoA transferase. Butyryl-CoA transferase pathway is dominant route for human colonic microbiota for butyrate synthesis[23].

SCFAis beneficial to prevent colon cancer (health of colon). In vitro assay reported SCFA could inhibit malignant cell line growth. Butyrate serves as a major nutrient for the intestinal epithelium cells, can inhibit proliferation, and induce apoptosis of colon cancer cells[21]. Fu et al.[24] reportedbydifferentiationmaker (cathepsin C), which showed that butyrate, propionate, and acetate could inhibit proliferation and motility of a well-differentiated human colonic cancer cell line. Purwani et al. [25] reported butyrate molar produced by fermentation *C.butyricum* BCC B2571 (2.6-5.2 mM) or *E. rectale* DSM 17629 (3.6-7.2 mM)inhibited proliferation and induceapoptosis of human colorectal cancer cell line HCT-116.

*3.5. Associations between microbes to SCFA concentrations*

*S*ignificant positive correlations were observed between concentrations SCFA (acetate, propionate, and butyrate) and type of microbe used: colonic microbiota or *C.butyricum* BCC-B2571 after 12 hours fermentation (Table 3). Fermentation RS by colonic microbiota indicated significant positive correlation with propionate concentration after 24 hours fermentation. Fermentation RS3 by *C.butyricum* BCC-B2571 indicated significant positive correlation with propionate and butyrate concentration after 24 hours fermentation. Our study, fermentation by *E. rectale* DSM 17629 observed one significant positive correlation with acetate after 24 hours fermentation.

## Conclusion

Hydrolysis by amilase-pullulanase increased RS content of IR-42 from 19% to 41% and Inpari-16 from 10% to 37%. Fermentation of RS3 IR-42 and Inpari-16 by colonic microbe, *Clostridium butyricum* BCC-B2571, and *E. rectale* DSM 17629, produced SCFA with different molar ratio. Time fermentation affected molar ratio of SCFA production. *C.butyricum* BCC-B2571 in medium supplemented with RS3 IR42 produced molar of acetate:propionate: butyrate, respectively 74.93 mM: 6.10 mM: 6.78 mM after 12 hours fermentation. Colonic microbiota in medium supplemented with RS3 IR42 produced molar of acetate:propionate: butyrate, respectively 63.28 mM: 6.27 mM: 6.84 mM after 24 hours fermentation. Both SCFA profile produced high butyrate molar than other profile. In our study, showed RS3 IR-42 had potential to produce butyrate.

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## References


[1] (2016). World Cancer Research Fund International. [08 Sept. 2016]. *http://www.wcrf.org/.*

[2] American Cancer Society, American Cancer Society. Colorectal Cancer Facts & Figures 2014-2016, Atlanta, Georgia, 2015.

[3] X. Wang, P.L. Conway, I.L. Brown, and A.J. Evans. In vitro utilization of amylopectin and high-amylose maize (amilomaize) starch granules by human colonic bacteria. *Appl Environ Microbiol.,* vol 65, pp. 4848-4854, Nov. 1999.

[4] (2014). Badan Pusat Statistik. [17 Feb. 2015].*https://www.bps.go.id/.*

[5] E.Y. Purwani, T. Purwadaria, and M.T. Suhartono, "Fermentation RS3 derived from sago and rice starch with Clostridium butyricum BCC-B2571 or Eubacterium Rectale DSM 17629", *Anaerobe*, vol 18 pp. 55-61, Sept. 2012.

[6] S.Y. Tan, "Resistant Rice Starch Development," M. Sc. thesis, Louisiana State University, Louisiana, USA, Augst. 2003.

[7] H.S. Guraya, C. James, and E.T. Champagne, "Effect of cooling, and freezing on the digestibility of debranched rice starch and physical properties of the resulting material", *Starch-Stärke*, vol53, pp 64-74, 2001.

[8] K.W. Kim, M.K. Chung, N.E. Kang, M.H. Kim, and O.J. Park, "Effect of resistant starch from corn or rice on glucose control, colonic events, and blood lipid concentrations in streptozotocin-induced diabetic rats," *J Nutr Biochem*., vol14, pp. 166-172, 2003.

[9] X.H. Zhao and Y. Lin, "Resistant starch prepared from high-amylose maize starch which citric acid hydrolysis and its simulated fermentation in vitro," *Eur Food Res Technol*.,vol 228, pp. 1015-1021, Feb. 2009.

[10] R. Sharp and G.T. Macfarlane, "Chemostat enrichments of human feces with resistant starch are selective for adherent butyrate-producing clostridia at high dilution rate," *Appl Environ Microbiol*.,vol 66, pp. 4212-4221, Oct. 2000.

[11] J. Yang, I. Martínez , J. Walter, A. Keshavarzian, and D.J. Rose, "In vitro characterization of the impact of selected dietary fibers on fecal microbiota composition and short chain fatty acid production," *Anaerobe*, vol 23, pp. 74-81, Jul. 2013.

[12] L. Wang and Y.J. Wang, "Rice starch isolation by neutral protease and high-intensity ultrasound," *J Cereal Sci*., vol39, pp.291-196, 2004.

[13] K.W. Kim, M.K. Chung, N.E. Kang, M.H. Kim, and O.J Park, "Effect of resistant starch from corn or rice on glucose control, colonic events, and blood lipid concentrations in streptozotocin-induced diabetic rats," *J Nutr Biochem*.,vol 14, pp. 166-172, 2003.

[14] AOAC, Official methods analysis, Washington DC: Association of Official Analytical Chemistry, 2006.

[15] B.O. Juliano, "A simplified assay for milled-rice amylose," *Cereal Sci Today*., vol 16, pp. 334-340, 1971.

[16] I. Goni, L. Garcia-Diz, E. Manas, and F. Saura-Calixto, "Analysis of resistant starch: a method for foods and food products," *Food Chem*., vol56, pp. 445-449, 1996.

[17] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, "Colometric method for determination of sugar and related substances." *J Anal Chem*., vol28, pp. 350-356, 1956.

[18] L.N. Panlasigui, L.U. Thompson, B.O. Juliano, C.M. Perez, S.H. Yiu, and G.R. Greenberg, "Rice varieties with similar amylose content differ in starch digestibility and glycemic response in humans," *Am J Clin Nutr*., vol 54, pp. 871-877, 19971.

[19] (2016). Indonesia Center for Rice Research. [20 August 2016]. Available : *www.bbpadi.litbang.pertanian.go.id*.

[20] U. Lesmes, E.J. Beards, G.R. Gibson, K.K. Tuohy, and E. Shimoni, "Effects of resistant starch type III polymorphs on human colon microbiota and short chain fatty acids in human gut model," *J Agric Food Chem*., vol 56, pp. 5415-5421, 2008.

[21] M. Waldecker, T. Kautenburger, H. Daumann, S. Veeriah, F. Will, H. Dietrich, B.L. Pool-Zobel, and D. Schrenk, "Histone-deacetylase inhibition and butyrate formation: fecal slurry incubations with apple pectin and apple juice extracts,"*Nutr.*, vol 24, pp.366–74, 2008.

[22] T.L. Miller and M.J. Wolin, "Pathways of acetate, propionate, and butyrate formation by the human fecal microbial flora",*App Environ Microbiol*., vol62, pp.1589-1592, 1996.

[23] P. Louis, S.H. Duncan, S.I. McCrea, J. Millar, M.S. Jackson, and H.J. Flint, "Restricted Distribution of the Butyrate Kinase Pathway among Butyrate-Producing Bacteria from the Human Colon",*J Bacteriol*., vol 186, pp. 2099-2106, 2004.

[24] H. Fu, Q.Y. Shi, and S. Jin, "Effect of short-chain fatty acids on the proliferation and differentiation

of the human colonic adenocarcinoma cell line Caco-2,"*Chin J Dig Dis*., vol 5(3), pp. 115–117, 2004.

[25] E.Y. Purwani, D. Iskandriati, and M.T. Suhartono, "Fermentation product of RS3 inhibited proliferation and Induced apoptosis in colon cancer cell HCT-116,"*Adv Biosci Biotech*., vol 3, pp. 1189-1198, 2012.

# Growth and Carcass Characteristic in Kampong x Broiler Crossbred Divergently Selected for Unsaturated Fatty Acid

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## Abstract

Unsaturated fatty acid is one of the group fatty acid composition which is important for human health. The objective of this study was to identify the growth and carcass characteristic in Kampong x Broiler crossbred chicken in divergent unsaturated fatty acid. The highest unsaturated fatty acid were devide into to two group (high and low), where high dan low sample had value were 58.87% and 50.43% respectively. The data were analyse using t-test to observe carcass characteristic between high and low unsaturated fatty acid. The result showed there were not significantly (P>0.05) differences in growth (body weight) and carcass characteristic (carcass weight, breast muscle weight, and leg muscle weight) between to selected group. Divergent selection base on unsaturated in Kampong x Broiler crossbred chicken could provide a usefull model for genetics studies of fatty acid content related trait.

Keywords : Broiler, carcass characteristic, kampong chicken, unsaturated fatty acid

## 1. Introduction

Kampong chicken is a native chicken from Indonesia. Kampong chicken is slow growth and lean meat type chicken but it has adaptability and resistancy to disease. Broiler is fast growth and fatty meat type chicken. Crossbreeding program hopefully can increase meat quality, growth, adaptability and resistancy. Fatty acid composition is closely related to the nutritive value and the teste of meat. Fatty acids play an important role in the component of meat quality such as tenderness, shelf life, and flavour [1]. Fatty acid were devide into saturated and unsaturated fatty acid. Saturated fatty acid such as C14:0 and C16:0 are risk factors for cardiovascular diseases [2]. In contrast, unsaturated fatty acids are beneficial to human health which have function to decrease the circulating consentration of low density lipoprotein (LDL)-cholesterol by increasing hepatic LDL receptor activity [3].

Unsaturated fatty acid are hydrocarbon chain containing at least one carbon-carbon double bond. In the UK, the major dietary sources of unsaturated fatty acids include meat and meat products. A well-established risk factor for cardiovascular disease is an elevated plasma low density lipoprotein (LDL) cholesterol consentration. Replacing saturated fatty acids with either monounsaturated fatty acids or n-6 PUFAs reduces LDL (the 'bad') cholesterol, and so reduces the risk of developing the disease. Unsaturated fatty acids, such as linoleic acid or monounsaturated fatty acids, also slightly raise high density lipoprotein (HDL) (the 'good') cholesterol, which assist in the removal of triacylglycerols from the bloodstream [4]. Previous studies have shown that diets rich in unsaturated fatty acids (UFA) led to lower fat content [5].

There is some evidence fat deposition such as intramuscular fat were are subject to

different regulatory mechanism such as growth, carcass characteristic and meat quality in chicken [6]. The aim of this study was to examine the consequences of divergent selection for unsaturated fatty acid content on growth and carcass characteristic in Kampong x Broiler crossbred chicken.

## 2. Material and Methods

### *2.1. Samples*

Tissue samples and phenotypes were collected from theKampong x Broiler crossbred chicken. Ten chicken were selected from a pool 62 chicken as according to unsaturated fatty acid value. Among the ten chickens use in unsaturated fatty acid study, five chickens were classified as extremely high and low unsaturated fatty acid level and consider for this study.

### *2.2. Fatty Acids Analysis*

Fatty acid analyis was determined by Soxhlet extraction method. Fatty acid analysis was done using Soxhlet method at Integrated Laboratorium at Bogor Agricultural University.

### *2.3. Statistical Analysis*

Differences between value from unsaturated fatty acid related to growth and carcass characteristic were analyse by the paired t-test (SAS 9.1). Value of $p<0.05$ were considered to indicate statisticaly significant differences. Pearson correlation coefficients were determined to know relationship between unsaturated fatty acidgrowth and carcass characteristic.

## 3. Result and Discussion

### *3.1 Unsaturated Fatty Acid Profile*

We focused on unsaturatedfatty acids that account for ~53 % ofthe total fatty acids (Table 1). The most abundant unsaturatedfatty acid was C18:1n9c, followed by C18:2n6c and C16:1, across the populations analyzed. C18:1n9c is an unsaturated fatty acid that is constitutes one third of chicken meat fat [7]. C18:1n9c was the major monounsaturated fatty acid comprising 26.71% in the yolk, whereas C18:2n6c was the major polyunsaturated fatty acid in the fatty acid content of quail egg yolk [8]. C16:1 and C18:1 are product that converted from C16:0 and C18:0 through the SCD enzyme catalyzes a Δ9-cis desaturation of a number of fatty acyl-CoA substrates with converted C16:0 to C16:1 and C18:0 to C18:1 [9].

### *3.2 Growth and Carcass Characteristic*

The level of unsaturated fatty acid were not found significantly ($P>0.05$) difference to growth trait (body weight) and carcass characteristic (carcass weight, breast muscle weight, and leg muscle weight) between to selected group (Table 2). Previous studies have shown that diets rich in unsaturated fatty acids (UFA) led to lower fat content (Sanz 2000) [5]. However, no difference between the content of IMF in leg [6] or breast muscle [10] was found between two lines divergently selected for abdominal fat percentage. Other researchers reported when using two types of essential unsaturated fatty acid (α-linoleic and α-linolenic) they observes differences which were highly significant ($P<0.01$) for carcass weight and the weight of the chest and thigh [11]. This means that even the type of fatty acid affect the quality of mass parts.

Table 1. Profile divergent unsaturated fatty acid

| Unsaturated Fatty Acid | Group | |
|---|---|---|
| | High | Low |
| C14:1 | 0.08 ± 0.02 | 0.08 ± 0.03 |
| C16:1 | 2.63 ± 0.87 | 2.28 ± 0.52 |
| C18:1n9t | 0.18 ± 0.02 | 0.12 ± 0.03 |
| C18:1n9c | 33.71 ± 0.94 | 30.04 ± 1.69 |
| C18:2n6c | 21.93 ± 2.26 | 19.39 ± 0.39 |
| C18:3n6 | 0.09 ± 0.01 | 0.08 ± 0.01 |
| C20:2 | 0.16 ± 0.03 | 0.18± 0.03 |
| C20:4n6 | 0.68 ± 0.21 | 1.12 ± 0.37 |
| C22:6n3 | 0.06 ± 0.02 | 0.11 ± 0.04 |

Table 2. Descriptive statistic of growth and carcass characteristics with divergent unsaturated fatty acid

| Carcass Characteristic | Group Unsaturated Fatty Acid | |
|---|---|---|
| | High | Low |
| Body Weight (g) | 1086.00 ± 180.88 | 1127.60 ± 162.30 |
| Chest Weight (g) | 202.40 ± 35.41 | 205.00 ± 36.28 |
| Carcass Weight (g) | 679.60 ± 101.95 | 707.20 ± 118.53 |
| Wing Weight (g) | 98.60 ± 9.29 | 95.20 ± 17.27 |
| Thigh Over Weight (g) | 122.20 ± 22.63 | 118.00 ± 22.44 |
| Thigh Down Weight (g) | 110.80 ± 17.31 | 121.20 ± 23.97 |
| Muscles Chest Weight (g) | 145.60 ± 25.72 | 141.40 ± 35.14 |
| Muscles Thigh Over Weight (g) | 92.20 ± 22.11 | 87.40 ± 17.39 |
| Muscles Thigh Down Weight (g) | 71.20 ± 12.56 | 77.00 ± 14.82 |
| Muscles Thigh Mayor Weight (g) | 104.40 ± 20.73 | 101.00 ± 27.10 |
| Muscles Thigh Minor Weight (g) | 41.80 ± 5.76 | 39.80 ± 8.93 |

Table 3. Correlation of growth and carcass characteristics with divergent unsaturated fatty acid

| Carcass Characteristic | Unsaturated Fatty Acids |
|---|---|
| Body Weight (g) | -0.03 |
| Chest Weight (g) | -0.07 |
| Carcass Weight (g) | -0.09 |
| Wing Weight (g) | 0.21 |
| Thigh Over Weight (g) | 0.15 |
| Thigh Down Weight (g) | -0.20 |
| Muscles Chest Weight (g) | 0.10 |
| Muscles Thigh Over Weight (g) | 0.13 |
| Muscles Thigh Down Weight (g) | -0.18 |
| Muscles Thigh Mayor Weight (g) | 0.12 |
| Muscles Thigh Minor Weight (g) | 0.11 |

*3.3 Correlation between Growth and Carcass Characteristics With Divergent Unsaturated Fatty Acid*

The correlation between unsaturated fatty acid with growth and carcass characteristic were variable with indication unfavourable correlation. Correlation analysis between growth and carcass characteristics with divergent unsaturated fatty acid are summarised in Table 3. The range of correlation were -0.20 to 0.21. The high correlation coefficients evident between unsaturated fatty acid with wing weight (0.21). The low correlation were between

unsaturated fatty acid with tight down weight (-0.20).Aldai *et al*. (2007) [12]reported positive correlations (P<0.001) were found between carcass conformation scores and unsaturated fatty acid (r=0.69) group.

## Conclusion

Unsaturatedfatty acid profile was C18:1n9c, followed by C18:2n6c, and C16:1, across the populations analyzed. The divergent of unsaturated fatty acid was not significantly (p>0.05)to growth and carcass characteristic between high and low sample. The correlation between unsaturated fatty acid with growth and carcass characteristic were variable with indication unfavourable correlation among the traits.

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## References

[21] Hoenselaar R., "Saturated fat and cardiovascular disease: The discrepancy between the scientific literature and dietary advice," J. Nutr, vol.28, pp. 118-123, 2011.

[22] FAO, "Fats and fatty acids in human nutrition," FAO Food Nutr. Pap, vol. 91, pp. 1-116, 2010.

[23] Taniguchi M, Utsugi T, Oyama K, Mannen H, Kobayasi M, Tanabe Y, Ogino A, Tsuji S, "Genotype of stearoyl-coA desaturase is associated with fatty acid composition in javanese black cattle," Mamm Genome, vol. 15, pp. 142-148, 2004.

[24] J. Lunn, and H.E. Theobald, "The health effects of dietary unsaturated fatty acids," *British Nutrition Foundation Bulletin.*, vol. 31, pp. 178–224, 2006.

[25] Sanz M, Lopez-Bote C.J, Flores A, Carmona J.M, "Effect of the inclusion time of dietary saturated and unsaturated fats before slaughter on the accumulation and composition of abdominal fat in female broiler chickens," Poult. Sci, vol. 79, pp. 1320-1325, 2000.

[26] Ricard F., Legleroq B., Tourable C, "Selecting broilers for low or high abdominal fat: distribution of carcass fat and quality of meat," British Poultry Science, vol. 24, pp. 511-516, 1983.

[27] Desnell, Zainal F, "Oxidation reaction kinetics myristic acid, stearic, and oleic in the medium coconut oil, palm oil, and without medium," Jurnal Penelitian Sains, vol. 12(1C): 12107 (1-6), 2009.

[28] Tokusoglu O, "The quality properties and saturated and unsaturated fatty acid profiles of quail egg: the alterations of fatty acids with process effects," Int. J. Food Sci. Nutr., vol.57, pp. 537-545, 2006.

[29] Enoch HG, Catala A, Strittmatter P, "Mechanism of rat liver microsomal stearyl-CoA desaturase: studies of the substrate specificity, enzyme-substrate interactions, and the function of lipid," J. Biol. Chem, vol. 251, pp. 5095-5510, 1976.

[30] Sibut V., L.E. B.E., Tesseraud S, Godet E, Bordeau T, Cailleau-Audoun E, Chartrin P, Duglos M, Berri C, "Adenosine monophospate-activated protein kinase involved in variations of muscle glycogen and breast meat quality between lean and fat chickens," Journal of Animal Science, vol. 86, pp. 2888-2896, 2008.

[31] Kralik G, Sktic Z, Kusec G, Kadlec J, "The influence of rape seed/oil on the quality of chicken carcass." J. Anim. Sci, vol.48, no.2, pp. 77-84, 2003.

[32] Aldai N, A.I Najera, A. Martinez, R. Celaya, K. Osoro, "Correlation between carcass conformation and fat cover degree, and muscle fatty acid profile of yearling bulls depending on breed and mh-genotype," Livestock Science, vol.107, pp. 199-212, 2007.

# Resistance against *Salmonella pullorum*in IPB-D1 Crossbreed, Kampong and Commercial Broiler Chicken

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## Abstract

The aim of this research was to study the resistance against *S. pullorum*infection on IPB-D1 crossbreed, kampong and commercial broiler chickens. Chicken IPB-D1 is derived from a cross between a male line of F1 pelungx sentul (PS crossbreed) with a female line of F1kampongx broiler parent stock, Cobb strain(KB crossbreed). A total of 31 chickens consisting of IPB-D1 (11 birds), kampong (13 birds) and commercial broiler (13 birds) were used in this research. All of chickens are collection of Division of Animal Breeding and Genetics, Department of Animal Production and Technology, Faculty of Animal Science, Bogor Agricultural University (IPB). The experiment was designed using a completely randomized design.Biological assays of resistance indicator were measured, including leukocytes profile (leucocytes concentration and its differentiation, H/L value), and clearance test using *S.pullorum.* Data from all observations were statistically analyzed using analysis of varian. The result of this research showed that the value of leukocytes profile and body resistance parameters from IPB-D1 crossbreed and kampong chicken were not significantly different, but on both these chickens, these parameters were significantly different than those in commercial broiler chickens. The conclusion is the IPB-D1 crossbreed biologically have the same resistance against *S. pullorum* infection with the kampong chicken and it higher than commercial broiler chickens.

Keywords: resistance, *S. pullorum*, crossbreed, kampong chicken, broiler chicken

## 1. Introduction

National meat consumption from poultry reached 66.97%. The needs of these poultry meat, still supplied by commercial chicken industry that 90% of its production components, of both breed and feed were import based. The meat consumption from the local chickens have just reached 11.10% [1]. This is due to small farming do not have a breeding program which is structured. So that the local chicken farmer is very difficult to get the selected chicken for breeding. This condition is very opposed to the potential of Indonesia is very rich in genetic resources of local chicken, and Indonesia is one of the centers of domestication of chickens in the world [9].

In 2010 [5] stated that in Indonesia there are 31 local chickens that have been identified and characterized. The existence of genetic resources from 31 local chickens is very important because it has a genetic basis information for the development of chickens in the future. The development of local chicken industry through the selection and crossbreeding between the local chicken with broiler parent stock could be an alternative choice for produce superior composite chicken based on local resources.

Chicken IPB-D1 is derived from a cross between a male line of F1 pelung x sentul (PS crossbreed) with a female line of F1 kampung x broiler parent stock, strain Cobb (KB crossbreed). The uses of these chickens are

based on the premise that the pelung chicken has the large framework, potentially resulting in a lot of meat. Sentul chicken was a kind of local chickens that has high eggs production [9], while the kampong chicken has high resistance against infection of Salmonella sp. [10, 11]. The third of local chickens that used, as Indonesian local chicken in general, have a late growth rate. The effort to increase the local chicken growth is through crossbreeding with broiler chicken. This crossing is expected to obtain the heterosis effect [6]. The presence of 25% the broiler blood in IPB-D1, is feared could reduce its body's resistance, because broiler chickens are very susceptible to diseases infections. Therefore, this study was conducted to evaluate the resistance of IPB-D1 chicken and compare it with kampong chicken and commercial broiler chicken.

## 2. MaterialandMethods

*2.1. Animal Experiments and Rearing*

The study was conducted in field laboratory of Division of Animal Genetics and Breeding, Faculty of Animal Science, IPB. It used three kinds of chicken (11 birds of IPB-D1, 13 birds of kampong chicken, and 13 birds of commercial broiler chicken). Each kind of chickens were placed in pen (1.5x1.0 $m^2$). Every pen was equipped with feed, water and light bulbs (18 Watt). Each of chickens were numbered.

This study used feed of broiler commercial that contain 22-23% crude protein for broiler chicken. IPB-D1 and kampong chicken were given a mix of 60% commercial broiler feed and 40% rice bran (17% crude protein). Feed and water were given *ad libitum*. Every week chicken body weight were weighed. Observation was done until IPB-D1 and kampong chicken 12-weeks aged, and the broiler chicken until 5-weeks aged. When all three types of chickens 5-weeks aged, blood samples were taken for analysis of leukocytes profile and clearance test.

*2.2. Observation of Leukocytes Profile*

The concentrations of leukocytes and its differentiation were assessed by the Giemsa method [8] in PhysiologyLaboratory, Faculty of Veterinary Medicine IPB, as follows: 20 µL of chicken blood was dissolved in 380 µL of Turk solution (1 mL of 1% gentian violet in water, 1 mL glacial acetic acid, and 100 mL distilled water) using a micropipette. The total number of leukocytes present was calculated by counting all viable cells present on four areas located in four corners of the room count under a light microscope (100x magnification) and then multiplying by 50 to determine the concentration of each $mm^3$.

*2.3. Observation of Body Resistance*

Body resistance was detected in blood samples using the clearance test [3] in Bacteriology Laboratory,Faculty of Veterinary Medicine IPB. This method was used to look at normal bacterial (*S. pullorum*) population growth compared that of populations weregiven specific treatment. The treatment impact on bacterial growth was measured after incubating for 24-48 hours at 35±1$^o$C. Preparation of bacteria culture begins with the rejuvenation of culture in nutrient medium at a temperature of 36±1$^o$C for 18-24 hours and a sub-culture on Brain Heart Broth medium at a temperature of 36±1$^o$C for 18-24 hours.

*2.4. Statistical Analysis*

This study used a completely randomized design. The kind of chicken as a treatment (IPB-D1 crossbreed, kampong, and commercial broiler chicken). The following model wasused :$Yij = \mu + Ti + \varepsilon ij$ (Yij is the result observation, µ is the overall mean, Ti is the effect of the kind of chickens, and εij is the random residual effect). Data were analyzed using the GLM procedure of SAS 9.1.3 software (SAS Institute, Cary, NC, USA).

Table 1. Body Weight of IPB-D1, Kampong and Broiler Chicken

| Kind of chicken (n) | Age (week) | Body weight (g/bird) |
|---|---|---|
| IPB-D1 (11) | 12 | 1 514.57±57.42 |
| Kampong (13) | 12 | 921.67±61.23 |
| Commercial broiler (13) | 5 | 1 550.26±30.71 |

Table 2. Leukocytes Profile in IPB-D1, Kampong and Commercial Broiler Chicken

| Leukocytes profile | IPB-D1 (n=11) | Kampong (n=13) | Commercial broiler(n=13) |
|---|---|---|---|
| Leukocytes concentration ($x10^3/mm^3$) | 23.16±3.10a | 22.23±2.17a | 29.91±2.73b |
| Heterophiles (%) | 43.22±3.25a | 41.37±2.81a | 70.83 ±3.41b |
| Monocytes (%) | 5.07±0.73a | 5.17±0.97a | 5.20±1.11b |
| Lymphocytes (%) | 50.16±4.02a | 51.69±3.13a | 22.42±0.61b |
| H/L ratio | 0.86±0.29a | 0.80±0.14a | 3.16±0.25b |

Different letters in the same row means different significantly (P<0.05)

Table 3. The Result of ClearanceTest in IPB-D1, Kampong and CommercialBroilerChicken

| Kind of chicken (n) | The ability to kill *S.pullorum* (%) |
|---|---|
| IPB-D1 (11) | 99.21±0.83A |
| Kampong (13) | 99.38±0.48A |
| Commercial broiler (13) | 36.67±0.66B |

Different letters in the same column means significantly different (P<0.01)

## 3. Result andDiscussion

### *3.1. Body Weight*

Body weight of IPB-D1 and kampong chicken at 12-weeks aged, and broiler commercial chicken body's weight at 5-weeks aged were presented in Table 1. The IPB-D1 crossbreed that was reared until 12 weeks produced body weight that almost the same with broiler commercial chicken at 5-weeks aged. The kampong chicken that was given the same feed with IPB-D1 only produced the body weight less than 1 kg/bird. The chicken body weight at 12-weeks aged according [9] almost reached 1 kg/bird (pelung chicken), and about 714 g/bird for sentul chicken. This research result showed the presence of heterosis effect on growth character in IPB-D1, which is derived from crossing between pelung, sentul, kampong, and broiler chicken.

### *3.2. Leukocytes Profile*

The leukocytes profile observation included leukocytes concentration, differentiation of leukocytes (heterophiles, monocytes, and lymphocytes), and H/Lvalue (Table 2). This result shows that leukocytes profile between IPB-D1 and kampong chicken were not significantly different, but both of this chickens were different significant (P<0.05) than those at commercial broiler chicken. Data leukocytesprofile (except H/L value) werein the normal range for chicken [4]. It means that all chickens were used in this study no interference physiologically.

IPB-D1 and kampong chicken have higher lymphocytes percentage than its heterophiles percentage. It means that the IPB-D1 and kampong chicken have higher ability to produce the specific immune response with forming antibody specific. Meanwhile the broiler chickenwhich the higher heterophiles percentage was potentially overcome the disease with non specific immune responsethrough phagocytosis [12].

H/L value shows the ability to overcome heat stress. The higher of H/L value means the chickens will experience

higher stress at high environment temperature.As such IPB-D1 can adapt well to the tropical environment such as kampong chicken,and on the contrary with the broiler chicken.

*3.3. Body Resistance*

Body resistance in this study was detected based on the ability to eliminate *S. pullorum* bacteria when in their blood samples were challenged with this bacteria through clearance test(in-vitro). The result of it was presented in Table 3.

This result showed that after 30 minutes blood were challenged with *S. pullorum* ($6.8x10^{10}$cfu/ml), blood from IPB-D1 and kampong chicken could eliminate this bacteria untill more than 99%, and highly significant different ($P<0.01$) than commercial broiler chicken (36.67%). IPB-D1 and kampong chicken can more kill *S. pullorum* because their blood contain high lymphocytes percentage. It can produce antibody specific [2] to against *S. pullorum.* In addition, they also can eliminate these bacteria through phagocytosis by heterophiles.

## Conclusion

Based on this study, it could be conluded that: 1). IPB-D1 and kampong chicken have body resistance against *S. pullorum*higher significant different than broiler commercial chicken, 2). At 12-weeks aged, IPB-D1 produce higher body weight than kampong chicken, and almost the same with body weight of commercial broiler chicken at 5-weeks aged, 3). IPB-D1 can be developed as a composite chicken as meat producer.

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## References

[1] [BPS] BadanPusatStatistik. Statistik Indonesia. Jakarta (ID): BadanPusatStatistik Indonesia. 2015.

[2] Ganong WF. Buku Ajar FisiologiKedokteran. Ed 22. Terjemahan. Jakarta. Penerbit Buku Kedokteran EGC. 2008.

[3] George JJ. Bacteriogical Analytical Manual. Published and Distributed by AOAC International. 1998.

[4] Jain NC. Essential of Veterinary Hematology. Philadelphia. Lea &Febiger. 1993.

[5] Nataamijaya AG. Pengembangan potensi ayam local untuk menunjang peningkatan kesejahteraan petani. J Litbang Pertanian 29(4):131-138. 2010.

[6] Noor RR. Genetika Ternak. Jakarta. Penebar Swadaya. 2010.

[7] Ganong WF. Buku Ajar Fisiologi Kedokteran. Ed 22. Terjemahan. Jakarta. Penerbit Buku Kedokteran EGC. 2008.

[8] Sastradipraja D, Sri Hartini SS, Reviany W, Tonny U, Achmad M, Hamdani N, Regina S, Razak H. Penuntun Praktikum Fisiologi Veteriner. Bogor. Pusat Antar Universitas Ilmu Hayat IPB. 1989.

[9] Sulandari S, Zein MSA, Paryanti S, Sartika T, Astuti M, Widjastuti T, Sujana E, Darana S, Setiawan I, Garnida D. Sumberdaya genetic ayam lokal Indonesia. Keanekaragaman Sumber Daya Hayati Ayam Lokal Indonesia :Manfaat dan Potensi. Pusat Penelitian Biologi. Lembaga Ilmu Pengetahuan Indonesia. Bogor. hlm 45-104. 2007.

[10] Ulupi N, Muladno, Sumantri C, Wibawan IWT. Association of TLR4 Gene Genotype and Resistance Against Salmonella enteritidis Natural Infection in Kampung Chicken. Intern. J. Poult. Sci. 12 (8): 445-450. 2013.

[11] Ulupi N, Muladno, Sumantri C, Wibawan IWT. Study of Kampung Chicken Resistance Against Salmonella enteritidis Using TLR4 Gene as Marker. Intern. J. Poult. Sci. 13 (8): 467-472, 2014.

[12] Wibawan IWT, Retno DS. 2013. Intisari Imunologi Medis. Fakultas Kedokteran Hewan IPB. Bogor. 2013

# Effects of Wheat Leaf Noni (*Morinda citrifolia*) on Carcass and Production Quail Eggs (*Coturnix Coturnix Javonica)* in the Different Level Concentrate

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## Abstract

This study aimed to determine the effect of leaf meal noni (*Morinda citrifolia*) at different levels in the ration of the carcass quail (*Coturnix Coturnix Javonica*) and quail egg production. The design used was completely randomized design (CRD). The treatments were attempted is A0 (feed without the addition of flour noni leaf 0%), A1 (ration with the addition of flour noni leaf as much as 3%), A2 (ration with the addition of flour noni leaf as much as 6%), and A3 (ration with the addition of leaf meal noni as much as 9%). Parameters measured were carcass weight (g), carcass percentage (%), abdominal fat (%), egg production was calculated based on the hen day (%), egg weight (g) and feed conversion and Income Over Feed Cost (IOFC). Results of analysis of variance showed that 73.33 g A0, A1 78.33 grams, 71.67 grams A2, and A3 72.50 gr, provides no real effect (P> 0.05) on carcass weight of quail. Treatment 65.71% A0, A1 60.23%, 65.02% A2, and A3 62.98%, giving the effect was not significant (P> 0.05) on carcass percentage quail. Results were significantly different (P <0.05) in the treatment of 0.84% A0, A1 0.73%, 1.36% A2 and A3 0.74% against abdominal fat. Treatment of 9.46% A0, A1 6.92%, 8.38% and A3 5.40% showed results significantly different (P <0.05) to the weight of quail eggs. Treatment A0 145 gr, 155 gr A1, A2 151.67 grams and 147.5 grams A3 shows the results significantly different (P <0.05) to the weight of the mother quail when first laying. The results of feed conversion analysis to egg production showed significantly different (P <0.05) in the treatment A0=11,26, A1=14,89, A2=12,34 dan A3=17,59. Value Analysis IOFC Rp.112.473,53 A0, -, A1 Rp.122.424,80, -, A2 Rp.121.713,99, -, and A3 range Rp.110.341,11. The conclusion that the administration of noni leaf meal in the ration significantly different to the carcass and quail egg production.

Keywords: Carcass, production of quail eggs, quail

## 1.Introduction

Feed is one of the main factors that is very important in the growth of quail. High feed intake is a great potential for increasing carcass weight and quail egg production. The need for increasing egg consumption in the market, it is because the price is affordable and contains a lot of nutrients and protein (Ratnaningsih, 2004). Much research has been done to find alternative feed ingredients in the hopes obtained ration prices are cheap, easy to obtain, available on an ongoing. basis, free of chemical stimulant drugs (pharmaceutical antibiotics) that does not interfere with the health of poultry, as well as having a balanced nutritional value.

Various kinds of herbs are used by farmers in the diet or in the drinking water of cattle in an attempt to re-nature that aims to get meat products and eggs special as lower cholesterol, increase feed efficiency substitute for antibiotics, anti-parasitic, and increase en-

durance for the people of Indonesia.

Expanding the use of herbs as medicine caused by several things, such as chemical drugs are very expensive, natural medicines readily available and fairly cheap price One way that can be done to improve carcass weight is the provision of a herbal plant noni leaf. This herb has been known since long ago by the people of Indonesia as a medicine as well as to improve the body's metabolism. Based on research Bangun (2002) Noni juice can inhibit tumor cells by stimulating the immune system that indicate award herbs tend to increase the percentage of carcasses and quail egg production. The aim of research to determine the effects of leaf meal noni (*Morinda citrifolia*) at different levels in the ration of the carcass and egg production quail (*Coturnix Coturnix Javonica*).

## 2.Materials and Methods

Research has been conducted in the stable, a variety of farm Mix Farming Experience (MFE) Faculty of Animal Padangsidimpuan for 6 weeks, from September to October 2015. Livestock used are quail aged 3 days DOQ (Day Old Quail) of 120 tail regardless of gender (unsexing), cage used as many as 24 plots with a size of 30 x 30 x 30 cm filled by 5 mice DOQ each cage is equipped with lights, the feeding and drinking places.

The study design used was completely randomized design (CRD) with number 4 and repeat the treatment as many as 6. A0 (ration without addition of noni leaf powder 0%), A1 (Rations with the addition of noni leaf meal as much as 3%), A2 (Rations with Extra flour noni leaves as much as 6%), A3 (with the addition of flour rations noni leaves as much as 9%). Making the noni leaf meal with a selection of noni leaf dark green uniform. Noni leaf must be free of pests and plant diseases and not dry. Washing is done in water that flows so that dirt and dust on the leaves disappear. Cutting leaves done to facilitate the drying process in oven incubator. The width of the chopped leaves with attempted so that the leaves can be dried simultaneously. Noni leaf that has been chopped and then dried in an oven incubator at 60°C - 80°C. Drying is done to ease the grinding process. The dried leaves ground in a blender to a powder.

The resulting leaf powder sieved using a sieve with a size of 150 μm to separate between fine and coarse powder. The ration of the research consisted of fish meal, corn flour, bran, palm kernel cake, soybean meal, noni leaf powder, and minerals is given the appropriate treatment. The research variables include carcass weight, carcass percentage, abdominal fat, egg production, the average egg weight, feed conversion to the production of eggs, and IOFC (Income Over Feed Cost).

## 3.Results and Discussions

*3.1 Carcass*

Carcass weight is the livestock after deducting the cutting head, the blood and internal organs, namely the feet and feathers (Soeparno, 1994). According to Sugiharto (2005), quail carcass is meat along the bone after being separated from the head to the neck of the boundary, from the foot to the knee and cavity contents abdomen. Analysis variance showed that the administration of noni flour in rations with different levels at different quail are not real ( P> 0.05) on carcass weight can be seen in Table 3.1.

Statistical analysis showed that the percentage of abdominal fat on all treatments affected by the provision of noni leaf powder in different levels in drinking water indicates significantly different (P<0.05). Abdominal fat is one component of body fat, which is found in the abdominal cavity.

Rating is based on carcass cattle carcass weight and degree of fatty body.because the absorption process is expected to help digestion enzyme protein complex into a simple protein that may be digested by the enzyme protease digestive tract.

Table 3.1. Average of Carcass Result

| Treatment | Parameters | | | |
|---|---|---|---|---|
| | Carcass weights (gr) | Carcass Percentage (%) | Abdominal Fat (%) | Analysis IOFC (Rp/10kg) |
| A0 | 73,33$^{ns}$ | 65,71$^{ns}$ | 0,84$^{b}$ | 112.473,53$^{ns}$ |
| A1 | 78,33$^{ns}$ | 60,23$^{ns}$ | 0,73$^{b}$ | 122.424,80$^{ns}$ |
| A2 | 71,67$^{ns}$ | 65,02$^{ns}$ | 1,36$^{a}$ | 121.713,99$^{ns}$ |
| A3 | 72,50$^{ns}$ | 62,98$^{ns}$ | 0,74$^{b}$ | 110.341,11$^{ns}$ |

Description: : ab = Significant (P<0,05)
ns = Non Significant (P>0,05)

Table 3.2. Average of Quail Egg Production

| Treatment | Parameters | | |
|---|---|---|---|
| | Henday (%) | Egg Weight (g) | Conversion Rations |
| A0 | 9,4607$^{a}$ | 8,7954$^{a}$ | 11,261$^{b}$ |
| A1 | 6,9168$^{bc}$ | 6,9169$^{ab}$ | 14,891$^{ab}$ |
| A2 | 8,3750$^{ab}$ | 8,3751$^{a}$ | 12,341$^{b}$ |
| A3 | 5,4034$^{c}$ | 5,4035$^{b}$ | 17,599$^{a}$ |

Description: The letters that follow the states numbers were significantly different (P <0.05)

Giving noni leaf powder on quail can increase carcass weight and reduce abdominal fat. Efficiency of noni as anthalmentika to kill worms poultry and pigs has been demonstrated in vitro by Djauhari (2003). This plant is in powder form has been considered invulnerable body. The ability of the animal body to recognize and destroy foreign material deemed. Cells that have small structural abnormalities would be recognized as a foreign substance and destroyed by the immune system of the body. Immune system function to prevent the growth of tumor cells, the basic elements of the body distinguish between normal and foreign materials as well as defend itself free from invading microorganisms. Makanisme immune response occurs in the lymphoid organs, therefore it must be provided an environment for efficient interaction between lymphocytes, macrophages and antigen. Based on research Bangun (2002) Noni juice can inhibit tumor cells by stimulating the immune system that indicate Award herbs tend to increase the percentage of carcasses.

Based on the reception during the study visits of value analysis IOFC treatment Rp.112.473,53 A0, -, A1Rp.122.424,80, -, A2 Rp.121.713,99, -, and A3 range Rp.110.341,11. This suggests that the animals were given rations of flour treatment noni leaf in the diet at the level of 3% treatment A1 provides real difference between the value of other OFC. IOFC analysis of results showed that the sale price of quail in kg live is still higher than the cost incurred ration

*3.2 Quail Egg Production*

From Table 3.2 it can be seen that the provision of flour noni leaf in rations with different levels at treatment A0 (ration without giving flour noni leaf 0%), treatment A1 (feed by administering flour noni leaf 3%), A2 (feed by administering flour noni leaf 6%), and A3 (feed by administering flour noni leaf 9%) give significantly different with egg weight (g / head / day), the weight of the parent when laying the first (g), feed conversion to the production of quail eggs (*Cortunix Cortunix Javonica*).Production of

eggs obtained in this study are still relatively low compared to some of the research that has been conducted shows that peak production is reached quail at the age of 65-70 days is estimated at 82-85%. According to Varghese (2007) that quail eggs a female can produce 200-300 eggs per year. Based on the results of analysis of variance, the effect of treatment giving noni leaf meal in rations with different levels showed significantly different (P <0.05) based on hen day egg production. Egg production is calculated based Hen Day (%) can be seen in Table 3.2. According Rasyaf (1983) high and low production of quail eggs on disebkan by variations quail preserved, namely their differences, maintenance, food, as well as a way of feeding the herbal one of them by giving noni leaf powder.

The effect of treatment provision Megkudu leaf meal in rations with different levels are significantly different (P <0.05) on egg weight. According to Noor (2000) heritability of egg weight is 0.60, which means egg weight has the properties inherited by the parent high, while according to Etches (1996) egg weight has a high heritability of about 0.45 to 0.85. The value of the average weight of the eggs results of the study are presented in Table 3.2.

Feed conversion ration the amount consumed is compared with the production of eggs produced. Factors affecting the quality of rations, delivery techniques, shapes and feed consumption (Amrulloh, 2003). Based on the results of analysis of variance, the effect of treatment provision Mengkudu leaf meal in rations with different levels are significantly different (P<0.05) feed conversion to the production of eggs. Extra noni leaf meal in the diet decrease feed conversion to the production of quail eggs. Research results with a further test showed that the average for each treatment produces 11.26 A0, A1 produces 14.89, A2 produces 12.34 and 17.59 A3 produces shown in Table 3.2.

## Conclusions

Based on the results of the study that administration of noni leaf powder at 3% level in the ration significantly different to the results of carcass weight, carcass percentage, and quail egg production. Suggestions needed for further studies of egg quality, such a large egg, yolk color and Haugh Unit value.

## References


Djauhari E. 2003. Mengkudu (Morinda citrilia) Tanaman Obat Potensial. Perkembangan Teknologi TRO. 15(1):17-28.

Endjo Djauhariya dan Agus Ruhnayat. 2002. Bercocok Tanam Mengkudu (Morinda citrifilia Li). Balai Penelitian Tanaman Rempah dan Obat,Bogor.

Hardjosworo, P.S. 1992. Berternak Puyuh. Fakultas Peternakan IPB, Bogor.

Junaedi . M. 2002. Burung Puyuh . UNDIP. Semarang.

Listiyomati,E., dan Kinanti, R., 2005. Puyuh Tata Laksana Budidaya Secara Komersial.Penebar Swadaya.Jakarta.75: 522–528.

Leeson, S., L. Caston dan J.D. Summer. 1996. Broiler Response to Energy and Protein Dilution in the Finisher. Diet. Polut. Sci.

Murata, K.H, I.Y. Edani and K. Koyanagi. 1967. Studieson The Nutritional Value of Tempeh. Food and Nutrition laboratory. Faculty of Sciene of Living. Osaka City University. Japan

Nugroho dan I.Gst.K.Mayun. 1986. Berternak Burung Puyuh. Eka Offset, Semarang.

Raden Enen Rina Rosadi Manggung. 2003. Pengaruh Pemberian Sari Buah Mengkudu (Morinda citrifolia Linn) Terhadap Pembentukan Folikel Limfoid Dalam Bursa Fabricus pada Embrio Telur Tertunas. Skripsi Fakultas Kedokteran Hewan Instutut Petanian Bogor. 2003.

Ribka, Dewi. 2011.Pengaruh Pemberian Daun Mengkudu dan Daun Nimba terhadap Rayap (Coptotermes curvignathus) (Isoptera; Rhinotermi) Di Laboratorium, Skripsi, Universitas Sumatera Utara, Medan.

Sugiharto, R.Eddy.2005. Meningkatkan Keuntungan Beternak Puyuh. Agromedia Pustaka. Jakarta

Susanto, T. dan B. Saneto, 1993. Teknologi Pengolahan Hasil Pertanian. Bina Ilmu,Surabaya.

Sutoyo.MD.1987. Petunjuk Praktis Beternak Puyuh. Titik terang. Jakarta.

Tetty.2002. Puyuh : Si Mungil Penuh Potensi. Cetakan Kedua.Jakarta. Penerbit PT. Agro Media Pustaka.

# Microbial Analysis on Freshwater Shell (*Corbicula sumatrana*) in Singkarak Lake Solok District West Sumatra

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## Abstract

*Corbicula sumatrana* is a shellfish which known by people with the name Lokan. Lokan consumed by people as a source of animal protein and animal feed ingredients. Singkarak Lake is used by people in daily life from the source of drinking water, toilets, fisheries, irrigation, Hydroelectric Power Plant and Tourism. Many activities of the community could be expected to cause pollution and affect the *C. sumatrana* become vectors of biotoxin because his diet is filter-feeder. Lokan meat is an excellent medium for bacterial growth. This study aims to determine the presence of *Escherichia coli* and *Salmonella* sp. on fresh meat shelfishes (*C. sumatrana*) originating from Singkarak Lake. This research is a descriptive method, by looking at and analyzing the presence of microorganisms are bacteria *E. coli* and *Salmonella* sp. The method used to determine the bacteriological quality of the meat is Lokan by MPN method and examination of *Salmonella*. Data were analyzed by calculating the number of bacteria *E. coli* by observing the number of positive results of the estimation of presumptive test, confirmative test and completed test. The next number of bacteria from each positive results are matched with MPN table. *Salmonella* sp. Test was done by looking at the colony grows. The results of bacteriological tests on meat samples were examined in BAPELKES Padang showed that of the four samples tested, three of which were negative for the bacteria *E. coli* samples take in Nagari Singkarak and samples contained negative for *Salmonella* sp. Of this study is suggested to consumers that cooking shellfishes perfectly, to avoid diseases that may occur because it is caused by bacteria.

Keywords: Bacterium, Meat, *Corbicula sumatrana*

## **1.** Introduction

Pelecypoda is the second largest class of the Mollusca Phylum which is widely used by the community as a source of animal protein or as raw material for industry [5]. In the waters of Indonesia live diverse types of Pelecypoda. There are live in fresh water (rivers and lakes) which are usually referred to shellfishes and clam that live in the sea. Utilization of animal protein sources have begun to demand by some communities in Indonesia, especially the types of Pelecypoda which has economic sense.

One of the animals that inhabit the bottom waters is *Corbicula sumatrana. C. sumatrana* is one of the benthic animals that inhabit the bottom waters which are muddy and sandy [5]. These types of shellfish harvested by people in large numbers. Because it is one of the types of foods that taste good. Residents around Singkarak Lake familiar with the term as pensi shells. Besides sold as pensi that are still intact, is also sold in the form without a shell. The shellfish is a source of practical and tasty food as a substitute for other animal protein.

Shellfish become vectors of biotoxin because the diet that are filter-feeders that by filtering food washed ashore or the flow of water through the gills and pass the necessary ingredients. This process causes the accumulation of plankton, chemical compounds and other small particles in the digestive tract shellfish [3]. Generally in Indonesia *C. sumatrana* is a typical freshwater mussels. *C. sumatrana* were also reported in Diatas Lake, Dibawah Lake and rivers which is located around the lake.

Singkarak Lake is one of the largest lake which is located in West Sumatra. In Singkarak Lake is widely available *C. sumatrana* which is used by the public, beside the habitat of this biota, the lake is also used in everyday life, ranging from drinking water sources, sanitation, fisheries, irrigation, Hydroelectric Power Plant (HEPP) and tourism , The number of community activities at Singkarak Lake, it can contaminate the water. According to Alcamo (1995) [6] waters are a wide range of microorganisms and macroorganisms life. Between microorganisms and macroorganism will occur the interaction of them, such as the bacteria are symbiotically with organisms that live in the waters such as plankton, zooplankton, fish, shrimp and scallops.

Most mussels are marketed in the fresh state (do not get treatment) without packing, thus enhancing the development of aerobic bacteria due to contact with air. Aerobic bacteria which can grow is *Salmonella* and others. *Salmonella* is one of the causes of infectious diseases. The affecting factor among other things is poor sanitation hygiene plays an important role in the spread of the disease. *Salmonella* can grow on milk and processed products, shellfish, frozen eggs, meat and meat products. It needs to be examined for bacteriological testing fresh meat of C. sumatrana shellfish that comes from Singkarak Lake.

## 2.Materials and Methods

The sampling of *C. sumatrana* in Singkarak Lake was held in January-May 2016. Bacteriological test is carried out in the Laboratory of Microbiology, BAPELKES Padang. The using tools are autoclaves, incubators, scales, erlenmeyer flask, test tubes, Durham tube, spiritus light, a petri dish, a sterile pipette 5 ml and 10 ml, ose needle, measuring cup, stir bar, test tube rack, alcohol spray, scissors , cookers, filters, buckets, tweezers, mortil, refrigerator, digital cameras and stationery. The using materials are Lokan's meat, selenith broth, LB medium, BGLB, EMB, SSA, plastic bags, distilled water, paper labels, lighters, sticky tape and tissue.

This research was conducted through a descriptive approach. Shellfish samples was taken at Singkarak Lake with 2 times of the decision. Data analysis was done by counting the number of *E. coli* bacteria by examining the number of positive results from the test results estimation, confirmation and the finisher. Furthermore, the number of bacteria of each positive result which was matched the MPN table. Salmonella sp. Testing was done by looking at the growing colony. Then, the results of this analysis are compared with the Decree of the Minister of Marine and Fisheries Number: KEP.17 / MEN / 2004 [7].

## 3.Results and Discussion

The result of bacteriological test of *C. sumatrana* shellfish's fresh meat that comes from Singkarak Lake in the Laboratory of Microbiology, BAPELKES Padang. The results of the bacteria *Escherichia coli* in shellfish flesh can be seen in Table 1. Based on the results showed that the samples of freshwater mussel meat has been contaminated with *E. coli*. *E. coli* is a bacteria of family Enterobacteriaceae which is a normal inhabitant of the gastrointestinal tract warm-blooded animals such as humans and livestock that are in the feces.

Table 1. Presence of Esherichia coli in the test table improvers with MPN / 100 ml with Variety: 5 x 10 ml, 1 x 1 ml and 1 x 0.1 ml.

| Sample Code | **T1** | **T2** | **T3** |
|---|---|---|---|
| A1 | - | + | - |
| A2 | - | - | + |
| A3 | - | - | + |
| A4 | - | - | - |
| B1 | + | - | + |
| B2 | + | + | - |
| B3 | - | - | + |
| B4 | - | - | - |

Description: T1 = 10 ml of the gas bubble tube, T2 = 1 ml of the gas bubble tube, T3 = 0.1 ml of the gas bubble tube, A = Location I, B = Location II

Table 2. Results of examination of bacteria *Salmonella* sp. reviewed Fresh Meat of shells that comes from Singkarak Lake.

| Sample | *Salmonella* sp. |
|---|---|
| L1 | Negatif |
| L2 | Negatif |

Description: L1 = Location I, L2 = Location II

If it is found that bacteria can be used as an indicator that the freshwater mussel meat contaminated by feces both humans and animals. The bacteria sanitation indicators generally are bacteria prevalent and live in the human gut, so the presence of the bacteria in water or food indicates that the sample had contact with feces from the human intestine and therefore it may contain other pathogens which are harmful.

According to [2], *E. coli* bacteria grows at temperatures of 10$^{o}$C to 40$^{o}$C and can die on heating above 40°C for 60 minutes. In Table 2 can be seen that the results of *Salmonella* sp. in *C. sumatrana* is negative on meat. *Salmonella* bacteria thrives in the intestinal tract of humans and animals and can cause food poisoning. Any raw food of animal origin, such as meat, eggs, milk and seafood may carry Salmonella bacteria [1].

Most of contamination of Salmonella sp. derived from feces. One of the diseases caused by Salmonella sp is enteric fever. This syndrome is caused by only a few Salmonella, the most important is S. typhi (fever typhoidal). Salmonella is ingested reach the small intestine into the lymphatic flow and then enter the bloodstream. The organism is carried by the blood to various organs, including the intestines. Salmonella multiply in limfoidusus tissue and excreted in the feces [4]. Based on the test results of Salmonella sp. means that the freshwater mussel meat is healthy for consumption by the consumer. As there was no bacterium Salmonella sp on freshwater mussel meat.

The mussels are filterfeeder which can accumulate bacteria contained in the habitat. Because it is a filter feeder and live as benthic animals, these shells are able to accumulate bacteria on meat [8]. Shellfish is one of fishery products which have important economic value. Generally shellfish, after capture of waters is not handled properly. The product quality of shellfish are highly influenced by the waters where shellfish are caught. Some evidence suggests that outbreaks of disease occur in humans due to eating contaminated shellfish. The types of diseases that comes from contaminated shellfish include; Salmonellosis, Tiphoid, Gastro-enteritis, Vibrio and biological toxins.

## Conclusion

Based on the results and discussion which are described above, it can be concluded that the mussel meat of *C. sumatrana* been contaminated with E. coli bacteria, but th*e Salmonella* sp. bacteria was not found on this clam meat. Based on the results of bacteriological test can be recommended that the freshwater mussel meat is healthy for consumption by the consumer.

## References

[1] Buckle, K. A. 1987. Food Science. Translators Hari Purnomo, Adiono. UI-Press: Jakarta.

[2] Entjang, Indan. 2003. Microbiology and Parasitology For the Academy of Nursing and School of Health Workers are equivalent. Citra Aditya Bakti: Bandung.

[3] Hikmah, A. 2011. Isolation and Identification of Vibrio cholerae bacteria in mussels in the fish auction place, region Sidoarjo. Scientific articles. Faculty of Veterinary Medicine, Airlangga University Surabaya.

[4] Jawetz, M. A. 2005. Medical Microbiology (Medical Microbiology), Salemba Medika: Jakarta.

[5] Kastoro, W. W. 1992. Some Aspects of Biology and Ecology of Marine Mollusk types needed to support the Commercial Cultivation. Proceedings of the Scientific Meeting of Resource Potential-Shellfish Shellfish South Sulawesi and Southeast Sulewesi. Maros Coastal Aquaculture Research Institute.

[6] Marlina. 2009. Identification of Bacteria Vibrio parahaemolitycus with Method Biology and Gene Detection by PCR ToxR her. Journal of Pharmaceutical Science and Technology. Vol. 13, No. 1.

[7] Regulation of the Minister of Marine and Fisheries. KEP.17 / MEN / 2004. Sanitation systems oyster Indonesia.

[8] Rachmawaty. 2008. Assessment of the microbial biofilter mussel (Corbicula sp) Journal of Biology, State University of Makassar. Vol. 9 (1): Pg 10-13, April 2008. ISSN: 1411-4720.

# Analysis of Estradiol and Progesterone Hormone Levels Against Various Cell Culture in TCM- 199 Medium for Cattle *In vitro*

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## Abstract

This research was aimed to obtain data base reproductive hormonal profile of the hormones estradiol and progesterone levels in various cell cultures. Culture cells used are cells fallopian tubes, ampulla, isthmus and follicle cells, whereas the culture period used were 0, 2 and 4 days. Analysis of the hormones estradiol and progesterone levels in various cell culture used ELISA method. Data results obtained are the estradiol hormone levels in various cell cultures and periods of different cultures in TCM-199 medium ie cell treatment Fallopian tubes in culture period 0, 2 and 4 days (9.07; 13.14; 9.00 pg/ml), cell culture period ampulla at 0, 2 and 4 days (9.00; 9.29; 14.39 pg/ml), cell isthmus (9.00; 12.08; 9.00 pg/ml) whereas follicular cells in culture period 0, 2 and 4 days (415.04; 476.67; 376.93 pg/ml). The highest levels of the hormone estradiol on cell cultures, namely follicle cells on the second day culture period (476.67 pg/ml), whereas the lowest in cell cultures, namely follicle cells on the fourth day culture period (376.93 pg/ml). Progesterone levels obtained in the treatment of Fallopian tube cells in culture period 0. 2 and 4 days (24.107; 24.644; 24.474 ng/ml), cell culture period ampulla at 0, 2 and 4 days (24.187; 23.753; 24.254 ng/ml ), cell isthmus (24.071; 24.083; 24.034 ng/ml) whereas follicular cells in culture period 0, 2 and 4 days (26.671; 27.610; 24.034 ng/ml). For progesterone levels in various cell culture and the culture that the treatment period follicle cell culture high on the second day culture period (27.610 ng/ml) and low progesterone levels in cell culture ampulla on the second day culture period (23.753 ng/ml).

Keywords: hormones, cell culture, medium TCM-199

## 1. Introduction

The cattleis one type of livestock that contribute greatly to meet the animal protein Indonesian society. It is estimated that demand for meat and milk in the future is increasing as a result of the growth of public awareness to consume animal protein.

The needs of people in Indonesia animal protein/ meat increased. In 2000, meat consumption is 1.72 kg/ capita/year by 2010, rising meat consumption of 2.72 kg/ capita/year or during the last 10 years people's needs for meat increased by 1.0 kg/capita/year (Victorbuana, 2010).

Beef cattle population data in Indonesia in 2011 amounted to 14,824,373 tail, whereas in 2015 the cattle population increased to 15,494,288 tail. Over the last 4 years the cattle population in Indonesia increased by 4.32% (Directorate General of Livestock, 2015). In response to the low increase in the cattle population it needs attention in the breeding of cattle.

Prospects of development of in vitro cell culture system is very large. This technique will be a lot to overcome fertility problems in humans and animals are facing the problem of infertility. During this time some of the technology used as *in vitro*maturation oocytes, in vitro fertilization and embryo transfer are based on in vitro cell culture systems, has been developed and

successfully applied with satisfactory results. Various culture systems have been developed in some species such as mice (Bishonga*et al*., 2001) and domestic animals (Miyano, 2005). Gordon (2003) suggests that the co-embryo culture supplemented several cell line like the Fallopian tube cells, cumulus and others can provide substances or growth factors necessary for embryonic development.

Reproductive process related to the mechanism of hormonal system, namely the relationship between hormones hypothalamic pituitary namely Gonadotrophin Releasing Hormone (GnRH), Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH), a hormone-ovarian hormones (estrogen andprogesteron) and hormone uterus (prostaglandins) (Hafez and Hafez 2000). Ovarian hormones that have a major role on reproduction are estrogen and progesterone.

Estrogen is a steroid hormone produced by the granulosa cells and theca cells of de Graaf follicles in the ovary. The main function is to stimulate the hormones estrogen estrus, stimulate the emergence of secondary sex characteristics, maintain a system of channels and the growth of female udder udders (Wodzicka-Tomaszewska*et al*., 1991).

According to Hafez and Hafez, (2000) that progesterone is one of the important hormones related to reproduction that is secreted by cells of Corpus Luteum(Cl).Clis an endocrine organ that is responsible for producing the hormone progesterone. Blood serum progesterone concentration can determine the state of the animal is in a state of infertility, normal, lust, and bunting so that it can be used for detection of estrus, pregnancy examinations and knowing other pathological conditions.

Cells Fallopian tubes play an important role in mammalian reproduction and can provide the optimal environment for oocyte maturation, capacitation of sperm, fertilization and transport of gametes and embryos, which is controlled by two sex hormone ovarian estrogen and progesterone (P4) (Leese*et al*., 2001); (Hunter, 2003).Co-cultured embryos with multiple cell line can provide substances or factors of growth necessary for the development embryos example cell oviduct, cumulus cells. (Gordon, 2003); (Trilaksana and Good, 2008). Furthermore, Hunter (2003) suggests that supplementation of cells tuba fallopian can increase cultured embryonic development.

Cells of ovarian follicles and *in vitro*culture can improve the development at a later stage, including oocyte growth, maturation and ovulation (Hartshorne, 1997). In vivo maturation process takes place in the follicle. In addition to the development and maturation, cell-cell follicles also actively produce steroid hormones such as progesterone and estrogen (Gordon, 2003). Gutierrez *et al*. (2000) suggests that the culture of follicular cells has important implications for the potential of biotechnology to produce a large number of oocytes for embryo transfer and development.

To improve the efficiency of production and reproduction in cattle, it would require an animal hormonal profile information (Katongole and Gombe, 2006). Many aspects of reproductive see cows have been investigated, but the profile information of estrogen and progesterone in a variety of cell culture (cell Fallopian tubes, ampulla, isthmus and follicular cells) for cell culture *in vitro* until now have not been reported.

Based on the above, the authors are interested in reviewing the hormonal levels in various cells as a research titled: "Analysis of Estradiol and Progesterone Hormone Levels Against Various Cell Culture in TCM- 199 Medium for Cattle *In vitro".*

The purpose of this study was to establish a baseline reproductive hormonal profiles of estradiol and progesterone hormone levels in various cells in the medium of oocyte maturation and embryo production *in vitro* and has benefits to provide information and the data about the profile of the reproductive hormones ( hormones

estradiol and progesterone ) in various cells as a reference to increase of the rate of oocyte maturation and embryo production cow in vitro in Efforts to Increase livestock numbers and assists in the rescue of germplasm and assist in providing breeding stock excels in bulk , quickly intervening and cheaply.

## 2. Materials and Methods

*2.1 Material Research*

Materials used in the study is the Fallopian tubes, ovaries cow that had been cut from Slaughter House (SH) Payakumbuh, West Sumatra. While the chemicals used are physiological 0.9% NaCl, Phosphate Buffer Saline (PBS), Tissue Culture Medium-199 (TCM-199; Sigma, M-5017), 10% fetal calf serum, gentamicin 50 ug / ml, FSH (Ovagen, Sigma), trypsin, mineral oil (M-8410, Sigma), streptomicyn-pennicillin (P-3539, Sigma), aquabidest, 70% alcohol, distilled water, and Kit estradiol hormone and progesterone. Furthermore, materials for co-culture cells used are cells Fallopian tubes, cell isthmus, ampulla cells and follicular cells.

The tool used is a pasteur pipette (fisher), Millipore filter 0.22 µm (Sigma), a petri dish Φ35 and Φ60 mm, brand Nikon microscope, $CO_2$ incubator, refrigenerator, an analytical balance Sartorius CP brand in 2245, ovens, laminar flow, razor blades, ovarian collection flask, $CO_2$ gas tube, Bunsen, centrifugation, micro tube and cover glass.

*2.2. Research Procedure*

Procedures for implementing the study are as follows:

*a. Ovary Retrieval of SH*

Ovaries were taken from cow ovaries SH is. After the last cut of beef cattle cleared from the ovarian tissue that covers the surface, then washed with PBS medium. Furthermore, the ovaries put in place that has been filled with a medium Physiological NaCl 0.9%. To avoid contamination by microorganisms in the medium Physiological NaCl0.9% was added streptomycine 0.1 mg / ml and penicillin 100 IU/ml and then stored in a thermos collection and ovaries were taken to the
laboratory at a temperature of 30-35$^0$C.

*b. The Oocyte Collection*

Oocyte collection is done with an incision technique/ Slicing is taking oocytes from the ovary to follicle-wrenching way on the surface of the ovary in the medium collection on a petri dish. Oocytes obtained from the collection and then put in a petri dish containing media collection. Media collection consists of Phosphate Buffer Saline (PBS) which was supplemented with 10% Fetal Calf Serum and gentamicin 50 mg/ml (Sigma, G-1397) that has been filtered using Millipore filter 0.22 µm.

*c. Making Cell Line for Cell Culture*

Fallopian tube tissue used was obtained from cow Fallopian tube that has been cut in the slaughterhouse. According Senger (1999) that the network started from the end of the Fallopian tube Fallopian tube that attaches to the cornua until the end that attaches to the infundibulum. Fallopian tube network isolation is done by inserting a medium D-PBS containing 0.25 % trypsin into the Fallopian tubes so that the cells of the Fallopian tubes fall out. Furthermore, the isolated cell is inserted into a petri dish, then isolated centrifuged for 10 minutes at a speed of 1800 rpm, 2 times. The precipitate obtained after setrifugation diluted with TCM-199 medium to a concentration of $10x10^6$ cells/ml. Furthermore Fallopian tube cells cultured in petri dishes in TCM-199 medium supplemented with FSH is at 10 µ/ml and gentamicin 50 g/ml and then incubated in a 5% $CO_2$ incubator at a temperature 38,5$^o$C up on the base of the petri dish to form a layer of cells line (*monolayer*).

For the treatment of isthmus tissue, obtained by cutting the Fallopian tubes cow on the isthmus. Senger (1999) reported that

the isthmus network starting from the end of the Fallopian tube that attaches cornua up with part of the Fallopian tubes which began to swell (ampulla). Isthmus network isolation is done by inserting a medium D-PBS containing 0.25 % trypsin into the cells of the isthmus loss. Furthermore, the isolated cell is inserted into a petri dish and then centrifuged for 10 minutes at a speed of 1800 rpm, 2 times. The precipitate obtained after setrifugation then diluted with TCM-199 medium to a concentration of $10x10^6$ cells/ml. Furthermore isthmus cells cultured in petri dishes in TCM-199 medium supplemented with FSH is at 10 µg/ml and gentamicin 50 µg/ml and then incubated in a 5% $CO_2$ incubator at a temperature 38,5$^oC$ up on the base of the petri dish to form a layer of the cell line (*monolayer*) .

Senger (1999) suggested that the network started from scratch ampulla enlargement until the end that attaches to the infundibulum. For the treatment of ampulla tissue is obtained by cutting the Fallopian tubes cow in the ampulla. Ampulla network isolation is done by inserting a medium D-PBS containing 0.25 % trypsin into the cells ampulla of the loss. Furthermore, the isolated cell is inserted into a petri dish and then centrifuged for 10 minutes at a speed of 1800 rpm, 2 times. The precipitate obtained after setrifugation then diluted with TCM-199 medium to a concentration of $10x10^6$ cells/ml. Furthermore ampulla cells cultured in a petri dish with TCM-199 medium supplemented with FSH is at 10 µg/ml and gentamicin 50 µg/ ml and then incubated in a 5% $CO_2$ incubator at a temperature 38,5$^oC$ up on the base of the petri dish to form a layer of the cell line (*monolayer*).

Making cell line (*monolayer*) of the follicle. Isolation of follicular cells used were obtained from flake when slicing follicles in the ovary. Isolated follicle cells put into a petri dish and then centrifuged for 10 minutes at a speed of 1800 rpm, two times.The precipitate obtained from centrifugation was diluted with TCM-199 medium to a concentration of $10x10^6$ cells/ml.

Furthermore follicle cells were cultured in a petri dish with TCM-199 medium supplemented with FSH is at 10 µg/ml and gentamicin 50 µg/ ml and then incubated in a 5% $CO_2$ incubator at a temperature of 38.5 °C at the bottom of a petri dish to form a layer cell line (*monolayer*).

*d. Analysis of Estradiol and Progesterone Hormone Levels In Various Cells Culture*

After the isolation of various cell culture treatment (cell fallopian tubes, ampulla, isthmus and follicles ) such as cell isolation techniques above. The isolated cells were cultured in a petri dish treatment for 0, 2 and 4 days. Each sample cell cultures treated for measuring levels of hormones estradiol and progesteron taken with a micro pipette and put into a micro tube. Samples treated cell cultures stored in a freezer for the collection of cell cultures and in the analysis of estradiol and progesterone hormone levels using ELISA.

*Variables Observed*

1. Levels of the hormone estradiol to a variety of cell culture (cell fallopian tubes, ampulla, isthmus and follicles) and a different culture period.
2. Levels of the hormone progesterone to a variety of cell culture (cell fallopian tubes, ampulla, isthmus and follicles) and a different culture period.

*e. Data Analysis*

Analysis of the data of the measured parameters presented descriptively displayed in the form of tables and figures.

## 3. Results and Discussion

*3.1. Hormone Levels of Estradiol*

Results of research estradiol hormone levels obtained in the treatment of a variety of cell culture supplementation and culture period in medium TCM- 199 are presented in Table 1.

Table 1. Hormone Estradiol Levels In Different Cell Culture andCulture Period (pg/ ml)

| No | Cells Culture | CulturePeriod (days) | | |
|---|---|---|---|---|
| | | 0 | 2 | 4 |
| 1. | Fallopiian Tube | 9,07 | 13,14 | 9,00 |
| 2. | Ampulla | 9,00 | 9,29 | 14,39 |
| 3. | Isthmus | 9,00 | 12,08 | 9,00 |
| 4. | Folicl1e | 415,04 | 476,67 | 376,93 |

Table 1 showed that the levels of the hormone estradiol in various cell culture and different culture period in medium TCM-199 is the highest Fallopian tube cell treatment on the second day culture period (13.14 pg/ml) followed by declines in 0 days (9,07pg/ml) and the fourth day showed the lowest levels of the hormone estradiol (9.00 pg/ml). Levels of the hormone estradiol on cell cultures ampulla and culture that the treatment period ampulla cell culture high on the fourth day culture period (14.39 pg/ml) was followed by a decrease in the second day (9.29 pg/ml) and at 0 days showed levels of the hormone estradiol the lowest (9.00 pg/ml). Levels of the hormone estradiol on cell cultures isthmus and culture that the treatment period isthmus cell culture high on the second day culture period (12.08 pg/ml) followed by declines in 0 days and the fourth day (9.00 pg/ml). Levels of the hormone estradiol in follicular cell culture that the treatment of follicular cell culture high on the second day culture period (476.67 pg/ml) followed by declines in 0 days (415.04 pg/ml) and the fourth day showed the lowest levels of the hormone estradiol (376,93 pg/ml).

The research results are obtained as shown in Figure 1 thatthe highest levels of the hormone estradiol follicle cell culture on the second day culture period (476.67 pg/ml) compared to the treatment of other cell culture, while the lowest estradiol levels in cell culture Fallopian tubes on the fourth day culture period (9.00 pg/ml), followed by cell culture ampulla of the culture period on day 0 (9.00 pg/ml) and in cell culture isthmus at the culture period on day 0 and day four (9.00 pg/ml).

This is due to the close ties of the follicular phase estradiol levels. According to Ganong (2003), estrogen will increase along with the development of follicles in the ovaries. Fluctuations in hormones estradiol in line with the development of follicles in the ovaries. When the development of follicles (follicular phase) of this hormone increases gradually, as the development of primary follicles into tertiary follicles. Estradiol hormone secretion peak occurs before ovulation occurs. Formed after ovulation and corpus luteum of the ovary (luteal phase), this hormone has decreased gradually until the end of the luteal phase.

High estradiol levels were predicted to be in the follicular phase, while cows that have lower estradiol content is predicted to be in the luteal phase. This is supported by Toelihere (1985) argues that the hormones progesterone blood is very high in the luteal phase and thus the activity of ovarian follicles in the growth of diminishing returns and as a result of the hormones estradiol further be low. In contrast to the follicular phase occurs around proestrus and estrus in estrous cycles showed that levels of the hormone estradiol in the blood high enough.

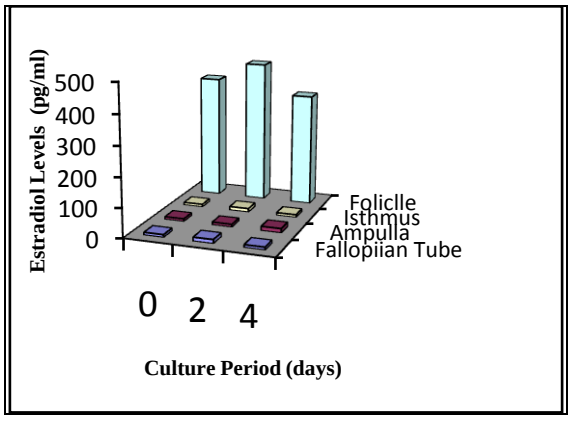


Figure 1. Hormones Estradiol Levels In Different Cell Culture and Culture Period

The research result was better than the results Saili*et al*. (2014), levels of the hormone estradiol in Bali cattle that ranged from 6.4 pg/ml up to 392 pg/ml. This is due to the levels of the hormones estradiol obtained from TCM-199 medium which was cultured in a variety of cultured cells. The follicle has a better percentage growth. Sirard and Coenen (1995) states that the culture medium, serum types that interact in the medium affects the growth and formation of groups of antral follicles *in vitro*.

According to McDowall *et al*. (2004) that the medium TCM-199 is often used as the basic medium for oocyte maturation *in vitro* because it contains elements of biochemical role in oocyte maturation. The addition of serum as a source of protein in TCM-199 is needed to support the process of oocyte growth. Fetal Bovine Serum (FBS) one supplement *in vitro* oocyte maturation medium used to stimulate the growth of a large number of cell cultures.

Levels of the hormone estradiol in various cell culture and the culture period is shown in Figure 1Showed that the treatment of follicular cell culture high on the second day culture period (476.67 pg/ml) and low estradiol levels in cell culture ampulla of the culture period on day 0 (9.00 pg/ml) in cell culture followed isthmus at the culture period on day 0 and day four (9.00 pg/ml). Lopez-Barbella*et al*. (1979) reported that the strong correlation between the concentration of estrogen at the time captivated by the number of CL.

Table 2. Progesterone Hormone Levels In Different Cell Culture and Culture Period (ng/ml)

| No | Cells Culture | Culture Period (days) | | |
|---|---|---|---|---|
| | | 0 | 2 | 4 |
| 1. | Fallopiian Tube | 24,107 | 24,644 | 24,474 |
| 2. | Ampulla | 24,187 | 23,753 | 24,254 |
| 3. | Isthmus | 24,071 | 24,083 | 24,034 |
| 4. | Folicle | 26,671 | 27,610 | 24,034 |

According to Dewi (2015), the ovaries secrete several reproductive hormones estradiol one of them is. Estradiol is a steroid hormone that plays an important role in reproductive status, while the most common components of hormone estradiol. Hormone estradiol serves to indicate the status of reproduction like effect on the reproductive system of female secondary sex characteristic and behavior of female lust. Follicular estradiol levels in small and large sizes are the same ranging from 1873.27 to 2012pg/ml.

*3.2. Progesterone Hormone Levels*

The results of the study progesterone levels obtained in the treatment of a variety of cell culture supplementation and culture period in medium TCM- 199 are presented in Table 2. Table 2 showed that the levels of the hormone progesterone in a variety of cell culture and different culture period in medium TCM-199 is the highest fallopian tube cell treatment on the second day culture period (24.644 ng/ml) was followed by a decrease in the fourth day (24.474 ng/ml) and day to zero indicate low progesterone levels (24.107 ng/ml). Progesterone levels in cell culture ampulla and the period of the culture that the treatment of cell culture ampulla highest culture period fourth day (24.254 ng/ml) was followed by a decrease in days to zero (24.187 ng/ml), and the second day showed progesterone levels low (23.753 ng/ml). Progesterone levels in cell culture isthmus and the period of the culture that the treatment of cell culture isthmus highest culture period the second day (24.083 ng/ml) followed by declines in 0 days (24.071 ng/ml) and the fourth day showed progesterone levels low (24.034 ng/ml).

From the research results that progesterone levels in various cell culture and the culture period showed follicular development have increased and decreased. Levels of the hormone progesterone in follicular cell culture high on the second day culture period (27.610 ng/ml) was followed

by a decrease to zero day (26.671 ng/ml) and the fourth day showed the lowest levels of the hormone progesterone (24.034 ng/ml). The increase in the hormone progesterone in the follicle cells occurs due to the LH peak that indicates the role of progesterone, while a decrease may occur due to follicles is already approaching the peak period of growth.

According Duria (2011) that progesterone is a hormone produced Cl. Added by Aparicio*et al*. (2011) found increased levels of progesterone in cultured two days due to the formation of CL occur after the follicles release an egg, so that the cows produce more CL and progesterone. The increase in the hormone progesterone in follicular cells occurred with short and quick, which is caused by the LH peak that indicates the role of progesterone.

Pahl*et al*. (2004) states that the large follicular growth rate is lower because of the antrum is fully formed so that the response to the lower medium. FolliCles with small size are more likely to experience growth due to the size of small follicles (2 - <4mm) in its infancy whereas large follicles almost reached the peak stage of growth (follicle *De graff*). *De graff* follicle growth rate is lower because of the antrum is fully formed so that the response to the lower medium.

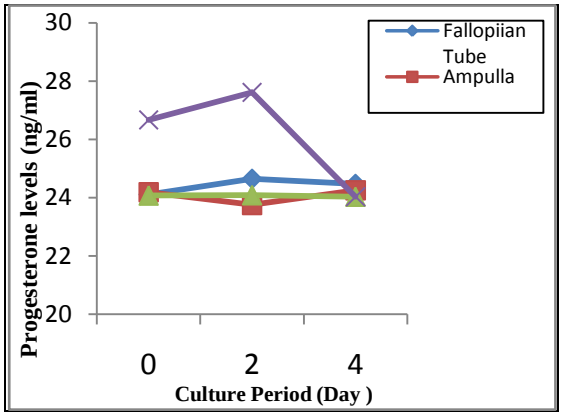


Figure 2. Progesterone Hormone Levels In Different Cell Culture and Culture Period

From the research that increased levels of progesterone occurs on the second day, this is due to CL. Confirmed by Cor (2014) points out that increased CL will increase production of the hormone progesterone. Siregar (2002) adds that the concentration of progesterone during the establishment period associated with the number of CL. Results of research Telfer*et al*. (2008) that the establishment of antral follicles were cultured happen quickly on a two-day culture.

According Ratnawati (2011) the hormone progesterone produced by CL and placenta. Progesterone levels in pregnant cow more than the cow is not pregnant. Progesterone occurs after ovulation and cause widespread development of the endometrium, uterus prepare to be ready to receive the embryo and feed. Broadly speaking, the physiological function of progesterone to the uterus of which block the effect of oxytocin on the myometrium, inhibit contraction of the myometrium and stimulate the growth of uterine glands in the endometrium.

The research results are obtained as shown in Figure 2 that the level of progesterone in cultured fourth day decline. This can happen because the follicle is approaching the peak of its growth and follicle cells are in the luteal phase. According to Tjiptosumirat (2009), when the ovaries do not contain CL, progesterone concentration decreases.

According Senger (2003) that the levels of progesterone in the eventual development of the follicle after luteolysis (without CL) and decreased levels of progesterone. Sariubang and Nurhayu (2011), when the cattle are already in the luteal phase means it has had a CL. As a result of these hormones reacted by lysing CL formed. This causes the lysis of CL progesterone levels decrease, resulting in loss of barriers to the hormone gonadotropin, which is followed by the growth and maturation of follicles, estrus and ovulation arise.

Profile of reproductive hormones during the cycle can describe the female ovarian function is concerned that analyzes hormone profile and its metabolites can indicate the condition of the female reproductive (Mostl and Palme, 2002). According to Siregar*et al*. (2004), the onset of estrus caused by lysis CL so that blood flow to the CL decreases dramatically. As a result, the levels of progesterone produced by CL will decrease. The decrease in progesterone levels will stimulate the anterior pituitary produces and releases FSH and LH. These hormones are responsible in the process of folliculogenesis and ovulation, resulting in the growth and maturation of follicles. Follicles eventually produce the hormone estrogen which is able to manifest the symptoms of estrus (Hafez and Hafez, 2000).

From the research that progesterone levels in various cell culture and the culture period in medium TCM-199 that the treatment culture follicular cells highest in the culture period the second day (27.610 ng/ml) and progesterone levels are lowest in cell culture ampulla of the culture period the second day (23.753 ng/ml). This is due to the development of CL during the estrous cycles that affect the levels of progesterone. CL formation has occurred after ovulation, which the hormone progesterone being produced. Conversely a decrease in progesterone levels occur after CL started to regress after estrus and began released luteolitik agent that can regressing CL. Hafez and Hafez(2000) suggested that CL regresses, causing reduced levels of the hormone progesterone.

Follicular growth can be influenced by the size of the follicles in culture, old culture and FBS. Goto*et al*. (1995) states that the culture conditions consist of the medium, the concentration of GH and use $CO_2$ incubator (long time culture) to support the growth of the follicle culture. Sirard and Coenen (1995) states that the culture medium, serum types that interact in the medium affects the growth and formation of groups of antral follicles *in vitro*. The length of time required for culturing follicles for 4 days, is intended to produce follicles and production of oocytes is better than the process of meiosis *in vitro*. Adam *et al*. (2004) reported that follicle consists of several core cells are coated by the cell membrane. The core of these cells have the potential to grow and become egg maturation occurs, if follicles fully developed, but there are several follicles did not develop and die then be replaced with new follicles.

## Conclusion

Based on the research results can be concluded is that the estradiol hormone levels in various cell culture and the culture that the treatment period follicle cell culture high on the second day culture period (476.67 pg/ml) and the lowest levels of estradiol on cell cultures in the period ampulla culture on day 0 (9.00 pg/ml) in cell culture followed isthmus at the culture period on day 0 and day four (9.00 pg/ml). Whereas progesterone levels in various cell culture and the culture that the treatment period follicle cell culture high on the second day culture period (27.610 ng/ml) and low progesterone levels in cell culture ampulla on the second day culture period (23.753 ng/ml).

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## References

1. Adam, A.A.G, Y. Takahashi, S. Katagiri and M Nagano. 2004. In vitro culture of mousepre antral folicle using membrane inserts and developmental competence of invitro ovulated oocytes. J. of Reproduction and Development Vol. 50: 579-586.
2. Aparicio. I. M., M. Garcia-Herreros, L.C. O'Shea, C. Hensey, P. Lonerganand T Fair. 2011.Expression, regulation, and function of progesteronereceptors in bovine cumulus oocyte

complexes during in vitromaturation. Biology of Reproduction 84 910–921.
3. Bishonga, C., Y. Takahashi, S. Katagiri, M. Nagano and A. Ishikhawa. 2001. *In vitro* growth of mouse ovarian preantalfolicles and the capacity of their oocytes to develop to the blastocys stage. Vet. Med. Sci. 63: 619-624.
4. Dewi,O. 2015. Levels of Estradiol on Fluid Cattle OvaryFollicles.http://etd.repository.ugm.ac.id/downloadfile/80423/potongan/abstract.pdf.
5. Directorate General of Animal Husbandry. 2015. Statistics of Animal Husbandry. Directorate General of Animal Husbandry, Jakarta.
6. Duria, N., B. Santoso, N. Lelananingtiyas and W.B. Santoso. 2011. Preparation of Samples for Measurement of Progesterone Cattle on Engineering Applications Radioimmunoassay. Prima. 8(1): 1411-0296.
7. Ganong, W.F. 2003. Review of Medical Physiology. International Edition. McGraw Hill Book. San Francisco.
8. Gordon, I. 2003. Laboratory Production of Cattle Embryos. Biotechnology in Agricultural Series. CAB. International.
9. Goto, K., T Yasuzuki, F. Watani and T. Shiniciro. 1995. *In vitro* development of bovine oocytes collected ovaries of individual cows after fertilization.J. Anim. Reprod. Sci.36: 110-113.
10. Gutierrez, C. G., J. H. Ralph, E. E. Telfer, I. Wilmut and R. Webb. 2000. Growth and antrum formation of bovine preantral follicles in long-term culture *in vitro*. *Biol. Reprod.*62(5): 1322-1328.
11. Hafez, B. and E.S.E. Hafez. 2000. Reproduction in Farm Animals. 7th ed. Lippincot Wiliams and Wilkins, Philadelphia.
12. Hartshorne, G. M. 1997. *In vitro* Culture of Ovarian Follicles. Reviews of Reproduction. PP. 94–104.
13. Hunter, R. H. 2003. Reflections upon sperm–endosalpingeal and sperm zonapellucida interactions *in vivo* and *in vitro*.Reprod. Domes.Animals. 3: 147–154.
14. Kor N.M. 2014. The effect of corpus luteum on hormonal composition of follicular fluid from different sized follicles and their relationship to serum concentrations in dairy cows. Asian Pac J Trop Med. 7(1): S282-S288.
15. Leese, H. J., J. I. Tay, J. Reischl and S. J. Downing. 2001. *Formation of fallopian tubal fluid: role of a neglected epithelium.* Reproduction.121*:* 339-*346.*
16. Miyano, T. 2005. *In vitro* growth of mammalian oocytes. J. Reprod. Dev.5:169 – 176.
17. Mostl, E and R. Palme. 2002. Hormones as indicator of stress. J. Domes. Anim. Endoctrinol. 23: 67 – 74.
18. Saili, T., A. Bain, A.S. Aku, M.Rusdin, and R. Aka. 2014. Estrus Synchronization Through Hormone Manipulation Luteolitik Agent to Improve Reproductive Efficiency of *Bali* and *PO* Cattle in Southeast Sulawesi. Department of Animal Husbandry, Agriculture Faculty, HaluoleoUniversity,Kendari.http://www.researchgate.net/publication/259849517.
19. Senger, P. L. 1999. Pathway to Pregnancy and Parturition. Current Concept Inc. Washington, USA.
20. Siregar, T.N. 2002. Progesterone Profile Measurement as a Method of Diagnosis of Early Pregnancy and The Birth of Twins at The Local Sheep. Media KedokteranHewan18(2):73-77.
21. Siregar, T.N., N. Areuby, G. Riady and Amiruddin. 2004. The Effect of PMSG against Ovarian Response and The Quality of Local Goat Embryo. Media Kedokteran Hewan 20(3):108-112.
22. Telfer E. E.,M. McLaughlin, C. Ding and K.J. Thong. 2008. A two-step serum-free culture system supports development of human oocytes from primordial follicles in the presence of activin. Hum Reprod.23(5):1151.
23. Toelihere, M. R. 1985. Physiology of Reproduction On Livestock. Angkasa, Bandung.
24. Victorbuana. 2010. Business Opportunities Beef Cattle. Peluang Usaha.web.id.
25. Wodzicka, T., M. J. K. Sutama, I. G. Putu and T. D. Chaniago. 1991. Reproduction, Behavior andAnimal Production in Indonesia.Gramedia Pustaka Utama, Jakarta.

# Acceleration Time Equilibration Cauda Epididymis Spermatozoa Buffalo with Addition of Antioxidant Gluthatione

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## Abstract

The purpose of this research is 1) Improving the quality of spermatozoa cauda epididymis buffalo during the equilibration process with the addition of the antioxidant glutathione 2) Efficiency equilibration time cauda epididymis the spermatozoa buffalo with the addition of the antioxidant glutathione. The parameters measured were the percentage of spermatozoa cauda epididymis buffalo during the equilibration process with the addition of the antioxidant glutathione. Percentage of cauda epididymis sperm motility buffalo during the equilibration process with the addition of the antioxidant glutathione. The percentage of abnormal spermatozoa buffalo cauda epididymis during equilibration process with the addition of the antioxidant glutathione. Research conducted an experiment using a randomized block design (RAK) 4 treatments and 6 replications time. Results of research cauda epididymis sperm quality buffalo during the equilibration process with the addition of the antioxidant glutathione with live sperm quality, motility and abnormalities in the 3 hour treatment is (85.33 ± 3.12), (77.5 ± 6:21) to (10:25 ± 1.17).

Keywords: equilibration, sperm, Cauda epididymis, glutathione

## 1.Introduction

The basic principle of the cryopreservation of spermatozoa is an expenditure of water from the cells before freezing intracellularly. If there is no water discharge will form ice crystals inside the cells and destroys the cells and experience drought so that the cells die (Supriatna & Pasaribu 1992). The principle of water displacement in and out of the cell membrane, either dehydration before deep freezing and dehydration at thawing should be noticed. Buffalo spermatozoa are sensitive to oxidative damage caused by high lipid peroxidation in the diluent. The process of freezing and thawing semen causes a decrease in sperm motility after thawing and lower conception rates (Lewis and Aitken, 2005).

A major factor in the cryopreservation of sperm cells which can reduce cell viability is the duration of equilibration, cold shock and changes in intracellular water due to excessive spending by the formation of ice crystals. An additional factor is the lipid peroxidation and antifreeze factors in seminal plasma as egg-yolk coagulating enzyme, lipase trigliserol and antimotilitas factor. To cope with damage to sperm cells during cryopreservation be accelerated equilibration time and the addition of glutathione (GSH) on cryoprotectants to improve the quality of spermatozoa during equilibration and cryopreservation.

## 2.Materials and Methods

This research was conducted at the Laboratory of Animal Biotechnology. Cauda

epididymis were taken from Slaughter House (RPH) for Padang Bandar. Part cauda epididymis buffalo collected with the technique of slicing, rinsing, and an emphasis on every network cauda epididymis (Rizal, 2006). The media used in the collection is the solution TALP. Furthermore, the concentration of spermatozoa were evaluated using a pipette hemacytometer and counting chamber, the percentage living with preparations using eosin staining with 3%, motility and abnormalities. Having in mind the quality of spermatozoa before diluted, hereinafter spermatozoa added glutathione 10% and included in the mini straw (0.25 ml) at a concentration of 30 million sperm cells using the syringe 5 ml by sucking. Spermatozoa in the mini straw given treatment time equilibration. P1 control 0 hours, P2 and P3 2 hours 3 hours in the refrigerator at 5 ° C. After equilibration process to evaluate the quality of spermatozoa in each treatment includes the percentage of survival, motility and abnormalities.

*2.1 Data Analysis*

Linear model design according to Steel and Torrie (1995) are:

$Yij = \mu + \tau i + \beta j + \Sigma ij$

Information :

$\mu$ = Mean common

$\tau i$ = Effect of treatment to i

$\beta j$ = observation group to j

Yij = Influence of residual / error in the experimental unit to j in treatment to i

i = Number of treatment

j = number of replicates / group

Data were analyzed using ANOVA (Analysis of Variance / ANOVA).

## 3.Results and Discussion

*3.1 Cauda Epididymis Sperm Quality Buffalo before Dilution*

The results showed the average concentration of cauda epididymal spermatozoa buffalo 246 million/ml ± 59.41 ml. Fresh sperm concentration obtained lower than stated Tappa 2007 ie 1.709 x 1.000.000. It is caused by a type of buffaloes and buffalo are also different from cattle. Percentage living a 88.80% ± 0.01%. This result is also different from the data published by Herrick et al., (2004) in African buffalo which amounted

to 92.75 ± 2.25%. In addition to the condition of the individual male buffalo, the percentage of spermatozoa cauda epididmis also influenced by a collection of spermatozoa after cattle in pieces. The longer the collection time epididmis the cauda spermatozoa, live spermatozoa percentage will decrease.

The percentage of fresh cauda epididymal sperm motility buffalo acquired a 0.01% ± 82.50% higher than the results Herrick et al., (2004), and Herold et al ,. (2004; 2006) to the African buffalo epididymal spermatozoa which amounted to 60.0 ± 3.82%; 58.0 ± 17.0% and 53.0% ± 12:51. This difference is due to different types of buffalo and individual conditions of each stud used in the study.

Table 1. Diversity Analysis Group Random Design (RAK)

| SK | DB | JK | KT | $F_{hitung}$ | $F_{tabel}$ 0.05 | 0.01 |
|---|---|---|---|---|---|---|
| Treatment | (3-1)=2 | JKP | JKP/2 | KTP/KTS | 3.33 | 5.64 |
| Group | (5-1)=4 | JKK | JKK/5 | KTK/KTS | 4.10 | 7.56 |
| rest | (t-1)(k-1)=8 | JKS | JKS/5 | | | |
| Total | (t.r-1)=14 | JKT | | | | |

The percentage of abnormal spermatozoa in fresh cauda epididmis is a 0.01% ± 9.80%. This result is not much different from that stated (Tappa, 2007) which is 11:31%. Percentage of cauda epididymal spermatozoa abnormalities buffalo influenced by genetics of livestock. Based on these results, further cauda epididymal spermatozoa buffalo processed with four treatments equilibration time control 0 hours, 1 hour, 2 hours and 3 hours.

*3.2 The quality of spermatozoa in the treatment of equilibration*

Quality of life is the best spermatozoa in equilibration treatment is treatment P4 (85.33±3.12), percentage motility P4 treatment (77.5±6.21) and abnormalities treatment P4 (10:25±1.17) with the results highly significant (P<0.01) than treatment P1, P2, and P3. The results of this study together with the results of research Umar and Maharani, 2005, that the longer the equilibration time improving the quality of life the percentage of spermatozoa with 82.17% and 47.17% in the treatment of motility equilibration 6 hours. The results also equal to the result Tuli et al. (1981) and Herdis et al. (1999) who get equilibration time of 4 hours resulted in the percentage of spermatozoa best buffalo compared to the equilibration time 2 and 6 hours. On the contrary Rao et al. (1990), Dhami and Kodagali (1990), Haranath et al. (1990), and Shiddique et al. (2006) advocated the equilibration time of 6 hours for buffalo spermatozoa.

GSH is known that the administration of 10% in diluent Cauda epididmis buffalo spermatozoa can improve the quality of life percentage, motility and decrease the number of abnormalities after equilibration in 3 hours at 5°C in the refrigerator. The role of GSH 10% because this concentration is an appropriate concentration and at this concentration the possibility of a balance between the binding reaction of free radicals by glutathione (GSH) which will be formed by the concentration of GSSG by the enzyme glutathione-peroxidase with GSSG into GSH by the enzyme glutathione reductase. This is supported by Kidd (1997) that the balance of the use of GSH by glutathione-peroxidase enzyme that will shape and change GSSG into GSH GSSG by the enzyme glutathione reductase regulated homeostasis. Furthermore, the equilibration occurs not only balance the concentration of glycerol, but other active components osmotic extenders (Salamon and Maxwell, 2000).

Table 2. Average of live sperm, motility and abnormalities after equilibration

| variables | treatment equilibration | value percentage |
|---|---|---|
| live spermatozoa | 0 Hour | 73.83 ± 13.07 |
| | 1 hour | 74.75 ± 8.88 |
| | 2 hour | 75.16 ± 12.02 |
| | 3 hour | 85.33 ± 3.12 |
| motile spermatozoa | 0 hour | 60.66 ± 0.00 |
| | 1 hour | 62.58 ± 2.88 |
| | 2 hour | 64.83 ± 4.12 |
| | 3 hour | 77.5 ± 6.21 |
| abnormal spermatozoa | 0 hour | 12.75 ± 0.61 |
| | 1 hour | 11.16 ± 2.22 |
| | 2 hour | 11.33 ± 2.06 |
| | 3 hour | 10.25 ± 1.17 |

Equilibration time difference will cause differences in the percentage of spermatozoa after freezing. In defending the state of sperm cells, glycerol takes considerable time to get into the cell membrane and keep the cell organelles from damage due to freezing spermatozoa. At the equilibration 5 hours of glycerol in the diluent is able to work optimally to inhibit the formation of ice crystals, thereby inhibiting the occurrence of the death of spermatozoa. Glycerol which binds to water molecules are free to form ice crystals are small and delicate, so it is not harmful to sperm cells. In addition, the equilibration time of 5 hours to allow sufficient time for the occurrence of potential chemical balance intracellular and extracellular water. At the equilibration time of 7 hours, not a proper balance chemical potential intra and extracellular water, so that when thawing damage will occur and the cell wall will cause the death of spermatozoa.

## Conclusion

The quality of spermatozoa in cryopreservation with GSH concentrations of 10% and in equilibration at 5°C showed the best results in the treatment of equilibration 3 hours. The role of GSH in diluent is balance between the binding reaction of free radicals by glutathione (GSH) with live sperm quality, motility and abnormalities in a row is (85.33 ± 3.12), (77.5 ± 6.21) to (10.25 ± 1.17).

## References

Dhami, A.J. and S.B. Kodagali.1990. Freezability, enzyme leakage and fertility of buffalo sperrmatozoa in relation to the quality of semen ejaculates and extenders. Theriogenology. 32(5):853-863.

Harananth, G.B.,T.B. Suryaprakasam,A.V.N. Rao, and G. Somasekharam, 1990. Freezability of semen and fertility of frozen semen packaged in mini and medium French straws: A note. Recent Advances in Buffalo Research.3:87-88.

Herdis, M.R. Toelihere., T.L Yusuf dan I. K. Kusuma. 1999. Integrity spermatozoa swamp buffalo (Bubalus bubalis) on various methods of freezing semen. JITV.4(1):7-12.

Herrick RF, McClean MD, Meeker JD, Baxter LK, Weymouth GA. 2004. An unrecognized source of PCB contamination in schools and other buildings. Environ Health Perspect 112(10):1051–1053.

Herold, F.C., K. De Haas, B. Colenbrander and D. Gerber. 2006. Comparison of equilibration times when freezing epididymal sperm from African buffalo (*Syncerus caffer*) using Triladyl™ or AndroMed®. *Theriogenology* 66: 1123-1130.

Lewis, S. E. & Aitken, R. J. (2005) DNA damage to spermatozoa has impacts on fertilization and pregnancy. Cell and Tissue Research 322, 33–41.

Rao, A.V.N., G.B. Haranath, and G. Somasekharam. 1990. Effect of equilibration and thawing rates on survival and acrosomal maintenance of buffalo spermatozoa in straws. Recent Advances in Buffalo Research.3:20-24.

Rizal, M. 2006. The influence of the addition of lactose in Tris extender on liquid semen quality Garut sheep. *J. Pengem. Petern. Tropis* 31: 224-231.

Salamon, S. and W. M. C. Maxwell. 1995a. Frozen storage of ram semen. I. Processing, freezing, thawing and fertility after cervical insemination. Anim. Reprod. Sci. 37:189-249.

Siddique M., R. Ali, and A. Raza. 2006. Effect of buffer on freezing of buffalo bull semen. J. Agricult. Soc. Sci.2(2):117-119.

Steel, R.G.D and Torrie. 1990. *Principles and Procedures of Statistics An Approach Biometrics. Interpreting Bambang Sumantri*. Gramedia Pustaka Utama, Jakarta.

Supriatna, I. Dan F.H. Pasaribu. 1992. In Vitro Fertilisation, Embryo Transfer and Embryo Freezing. Inter-university center, institut Pertanian Bogor,Bogor.

Tuli, R.K., M. Singh, and J.S. Matharoo. 1981. Fertility trial under field conditions with frozen buffalo bull semen using Tris yolk glycerol extender. Indian J. Dairy Sci. 34:456-458.

# Buffalo Embryo Maturation Optimization in Vitro with Addition Glutathione

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## Abstract

High fat content in the media buffalo oocyte maturation in vitro oocyte effect against oxidative stress. Increase oxidative stress resulted in low numbers maturation In vitro oocyte maturation. The aim of research to determine the concentration of the addition of glutathione (GSH) on media buffalo oocyte maturation in vitro. Increasing the percentage of water buffalo oocyte maturation in vitro. Increasing the percentage fertilization of buffalo oocytes in vitro. Materials used are buffalo ovaries with laboratory experimental method. The design is a randomized block design (RAK) GSH 4 treatments and 6 replications. The addition of 1.5 mM glutathione in the media buffalo oocyte maturation in vitro highly significant that the percentage of ripening (P <0:01) or 62.5% and the percentage of fertilized oocytes 88.98%.

*Keywords: Maturation, fertilization, oocytes, buffalo, in vitro*

## 1. Introduction

Aplikasi technology In Vitro fertilization (IVF) is to produce embryos are many and are an alternative to increase the population of buffaloes, increase productivity and genetic quality. Constraints application of In Vitro fertilization technology applications, namely during the maturation process of oocytes, fertilization and culture media composition is not conformity maturation oocytes, embryos with a culture medium compositions physiological conditions buffalo oocytes in maturation in vitro.

Physiologically, buffalo oocytes and embryos contain a high fat content during the process of maturation and embryos during in vitro culture (Boni et al., 1992). The concentration of high fat content in the medium maturation oocytes and embryos buffalo culture medium In vitro, influential and sensitive to an increase in oxidative stress that occurs in conditions of In Vitro (Gasparrini et al., 2003).

To reduce the oxidative stress caused by high fat content, it is necessary chemicals to reduce the risk. One of the chemicals that can reduce oxidative stress to cells in mammals and during the maturation In vitro culture is glutathione (GSH) (De Matos and Furnus, 2000).

Glutathione (C10, H17, N3O6S) and its derivatives which is a tri-peptide (y-Glu-Cys-Gly) can affect many aspects of metabolism, including helping detoxification and transport of y-glutamilamino acid, which is expected to increase the percentage of fertilization in vitro that can eventually increase the percentage of embryos up to the morula and blastocyst (De Matos and Furnus, 2000).

Not optimal success rate of technology FIV buffalo caused high levels of fat at the time of maturation and culture embryos in vitro, then made an effort to improve the success (FIV) with a variety of treatments such as addition of chemicals glutathione (GSH) with the appropriate concentration into the medium maturation of oocytes and the culture medium in vitro embryos.

## 2.Materials and Methods

This research was conducted at the Laboratory of Reproductive Physiology, Faculty of Animal Husbandry Universitas Andalas. Buffalo ovaries were collected from Slaughter House (RPH) as a source of egg cells (oocytes), immediately after the buffalo were cut using medium Phosphate-buffered saline (PBS) temperatures around 35°C in a flask. On arrival at the laboratory ovary washed with fresh PBS and placed in a beaker in a water temperature of 37°C. Of follicles <5 mm, oocytes aspirated with PBS medium using a 10 cc syringe and hypodermic needles size 18 G. After the ovary aspirated, made the incision to locate oocytes that may remain in the medium slicing ovary carefully and then sprayed with PBS medium and accommodated in a petri dish diameter of 5 cm. Oocytes were transferred in a 10 cm diameter petri dish using sterile pasteur pipette.

Oocytes were collected and selected under the microscope, and then in culture in maturation medium TCM-199 enriched with FSH, serum and gentamicin in a 5% CO2 incubator temperature of 38.5°C and 90% humidity for 24 hours. On maturation medium TCM-199, the addition of glutathione at a concentration of 0, 0.5, 1, and 1.5 mM as treatments A, B, C and D. Cement stud buffalo obtained from slaughterhouses Padang Bandar Create and collected in laboratory using a 10 ml syringe and the emphasis in each channel epididymis. Oocytes that have undergone maturation in each treatment A, B, C and D, washed with media TCM-199 twice and once in the medium fertilization, then placed in a medium Tyrode's albumin lactate pyruvate (TALP) 5 ml and injected into the medium fertilization with concentration of 1 x 1.000.000 cells spermatozoa (Triwulanningsih et al., 2001 a).

After the sperm incubated together for 18 hours in a 5% CO2 incubator, oocytes were washed with medium TALP 2 times to remove the cumulus cells surrounding oocytes still attached. Furthermore, oocytes were washed again in medium TALP one and count the number of fertilized oocytes in each treatment. The parameters observed in this study is the percentage meture buffalo oocytes in each treatment media Penamabahan glutathione (GSH) in vitro. Percentage of buffalo oocytes matured in the fertilized after each treatment In vitro maturation medium. The method used in this study is an experimental method and design used was a randomized block design (RBD) with three treatments and six replications as a group.

### *2.1 Data Analysis*

Linear model design according to Steel and Torrie (1995) are:

$Yij = \mu + \tau i + \beta j + \Sigma ij$

Information :

$\mu$ = Mean common

$\tau i$ = Effect of treatment to i

$\beta j$ = observation group to j

Yij = Influence of residual / error in the experimental unit to j in treatment to i

i = Number of treatment

j = number of replicates / group

Data were analyzed statistically using ANOVA (Analysis of Variance / ANOVA).

## 3. Results and Discussion

### *3.1 Percentage of buffalo oocytes were mature in treatment Penamabahan glutathione (GSH) in In Vitro*

The results showed that the highest percentage of buffalo oocytes were mature in 5% CO_2 incubator for 24 hours in TCM-199 medium are oocytes matured by the addition of 1.5 mM Gluthatione. The level of maturity of each treatment can be seen in Table 2.

The addition of 1.5 mM in the medium Gluthatione maturation of buffalo oocytes in vitro maturation generate percentages were significantly ($P < 0.01$) in higher than maturation with the addition Gluthatione 0 mM, 0.5 mM and 1.0 mM GSH. While the percentage of buffalo oocyte maturation with the addition Gluthatione 0 mM, 0.5 mM and 1.0 mM GSH showed no significant differences ($P > 0.05$). It is the addition of 1.5 mM Gluthatione in buffalo oocyte

Table 1. Analysis of Variance Randomized Block Design

| SK | DB | JK | KT | $F_{hitung}$ | $F_{tabel}$ 0.05 | 0.01 |
|---|---|---|---|---|---|---|
| Treatment | (4-1)=3 | JKP | JKP/2 | KTP/KTS | 3.33 | 5.64 |
| group | (6-1)=5 | JKK | JKK/5 | KTK/KTS | 4.10 | 7.56 |
| Rest | (t-1)(k-1)=15 | JKS | JKS/5 | | | |
| Total | (t.r-1)=2 | JKT | | | | |

Table 2. Buffalo Mature oocytes percentage in addition Glutathione (%)

| Treatment | N | The percentage of oocytes Buffalo Mature (%) |
|---|---|---|
| A (control, 0% Gluthatione ) | *213* | *30.49*[a] |
| B (0.5 Gluthatione) | *211* | *31.91*[a] |
| C (1.0 Gluthatione) | *211* | *42.23*[a] |
| D (1.5 Gluthatione) | 211 | *62.51*[b] |

maturation medium effectively enhance the success of buffalo oocytes maturation in vitro.

Percentage of maturation higher Gluthatione addition of 1.5 mM GSH for Gluthatione concentration of 1.5 mM effectively resist the oxidative stress caused by high levels of fat secreted by buffalo oocytes during maturation process. Further explained that GSH protects against oxidative damage found in two forms, namely the form of reducing (GSH) and oxidize form (GSSG). Glutathione protects against reactive oxygen species (ROS) in a manner facilitated by the interaction with enzymes such as glutathione peroxidase and glutathione reductase. In animal tissues, glutathione peroxidase is an antioxidant selenium-containing enzyme, speed reduction and lipid peroxidase hydrogen peroxide in the presence of GSH to be converted to GSSG.

Major et al., (2001) stated that the addition of 1 mM GSH in a maturation medium can increase the level of intracellular GSH (3.23 pmol / oocyte) compared with no addition and the addition of 0.25, and 0.5 mM on oocytes goat prepubertas. The concentration of GSH intracellular processes maturation of oocytes in vitro reflects the degree of maturation of the cytoplasm (Funahashi et al., 1994), and may be an indicator of value from the maturation of the cytoplasm (De Matos et al., 1997, Abeydeera et al., 1998, Furnus et al., 1998, De Matos and Furnus, 2000).

Intracellular GSH synthesis normally takes place during the maturation process so that the addition of exogenous GSH did not significantly affect the maturation level of the cell nucleus. De Matos et al., (2002) stated that GSH is synthesized during the process of maturation both in vivo and in vitro. Further stated that the concentration of intracellular GSH sheep oocytes were matured in vitro using medium TCM-199 ranged from 4.2 to 6.5 pmol / oocyte matured while in vivo the range of 6.38 ± 1.58 pmol / oocyte (Livingston et al., 2009). It shows that the concentration of GSH intracellular in oocytes of sheep were matured in vitro and in vivo is not much different, so the addition of GSH in medium maturation does not give effect optimally to increase the number of oocytes that reached the stage of metaphase II (MII), although it showed a trend increase in line with increased concentrations of GSH added.

Some researchers found that the addition of GSH may have a positive influence on cytoplasmic maturation so that it can support the formation of pronuclei in this study. The addition of GSH only in the culture medium results were better than the control, due to the

addition of GSH when culture may make the condition better culture than in control so that the number of pronuclei formed more. Karja et al., (2006) states that the decrease in GSH concentration in cells from unfertilized oocyte to develop into blastocysts. Kim et al., (1999) and Zuelke et al., (2003) explains that the oocytes mature, GSH has an important role in the formation of the male pronucleus after fertilization. Maedomari et al., (2007) stated that GSH is present in the oocyte cytoplasm plays a role in the formation of pronuclei beginning of the breakdown of the nuclear membrane disulfide bonds, followed by initiation dekondensasi chromosome.

No real effect maturation rate of buffalo oocytes in vitro in the treatment 0 mM, 0.5mm and 1.0mm GSH GSH is because the dose has not been able to reduce the concentration of fat and have not effectively resist oxidative sters caused by high levels of fat in the oocyte maturation media water buffalo in Vitro. Apart from the causes of the low rate of maturation and fertilization of buffalo oocytes in vitro in the treatment 0 mM, 0.5 mM and 1.5 mM GSH likely due to differences in individual buffal /ovaries as a source of oocytes. For every time a collection of ovaries from slaughterhouses, not all ovarian comes from buffaloes of the same age, but is derived from buffalo culled, buffalo too young or buffaloes are often used as labor, so the quality of the oocyte before being treated also varies which creates large diversity of results. This can be overcome by increasing the repetition (Triwulanningsih et al., 2002).

Gluthatione addition of 1.5 mM in medium buffalo oocyte maturation in vitro resulted in the percentage of fertilized oocytes were significantly ($P < 0.01$) in higher than maturation with the addition Gluthatione 0 mM, 0.5 mM and 1.0 mM. While the percentage fertilization of buffalo oocytes with the addition Gluthatione 0 mM, 0.5 mM and 1.0 mM showed no significant differences ($P > 0.05$). This means that the addition of 1.5 mM Gluthatione in buffalo oocyte maturation medium is effective to increase the success of fertilization of buffalo oocytes in vitro.

The results of these studies can be seen that the higher the success rate of oocyte nucleus maturation in vitro, will be a direct impact on the level of fertilization. It is suspected that the very important role Gluthatione additions made during maturation of buffalo oocytes in vitro. At the time of buffalo oocytes maturation in vitro, functioning Gluthatione suppress oxidative stress that is secreted by cells and oocytes during oocyte maturation so that when fertilized with cauda epididymal spermatozoa, the higher the success rate of in vitro fertilization makeover.

The percentage of fertilized oocytes were higher in the addition of 1.5 mM Gluthatione allegedly because Gluthatione very important role to reduce the oxidative stress of cells oocytes. The results are consistent with the results presented by Urdaneta et al., (2004) that the addition of 1.0 mM GSH in medium fertilization fertilization increase the rate of 10.61% to 30.20% compared with no addition of GSH in oocytes goat prepubertas. More Kim et al., (1999) also reported the addition of 1 mM GSH in medium fertilization, the number of embryos cows reached the stage of blastocyst was higher (27.3%) compared to 0 mM (20.1%), 0.1 mM (21.8%) and 10 mM (8.9%).

Furthermore Maedomari et al., (2007) stated that GSH is present in the oocyte cytoplasm plays a role in the formation of pronuclei beginning of solving the nuclear

Table 3. Percentage of buffalo oocytes fertilized in addition Glutathione (%)

| Treatment | N | The percentage of fertilized oocytes Buffalo (%) |
|---|---|---|
| A (kontrol, 0% Gluthatione ) | *65* | *33.43*[a] |
| B (0.5 Gluthatione) | *67* | *47.60*[a] |
| C (1.0 Gluthatione) | *89* | *48.50*[a] |
| D (1.5 Gluthatione) | *132* | *88.98*[b] |

membrane disulfide bonds, followed by initiation dekondensasi chromosome. Further Zuelke et al., (2003) explains that the oocytes mature, GSH has an important role in the formation of the male pronucleus after fertilization.

## Conclusion

The addition of glutathione 1.5 mM in medium maturation oocytes buffalo in vitro provide a significant influence on the percentage of maturation ie (P <0:01) or 62.5% and in line with the high percentage of oocytes matured in treatment increase glutathione 1.5 mM, then the percentage of oocytes were successfully fertilized also getting high at 88.98%. Abeydeera LR, Wang WH, Cantley TC, Rieke A, Day BN. 1998. Coculture with follicular shell pieces can enhance the developmental competence of pig oocytes after in vitro fertilization: Relevance to intracellular glutathione. *Biol Reprod* 58: 213-218.

## References


Boni, R., Sangella, L., Dale, B., Rovello, S., Di Palo, R., Barbieri, V., 1992. Maturazione in vitro di oociti bufalini: indagine ultrastrutturale. Acta Med. Vet. 38:153-161.

De Matos DG, Furnus CC, Moses DF. 1997. Glutathione synthesis during in vitro maturation of bovine oocytes: Role of cumulus cells. *Biol Reprod* 57: 1420-1425.

De Matos D.G. and C.C.Furnus. 2000.The importance of having high glutathione (GSH) level after bovine in vitro maturation on embryo development. Effect of beta-mercaptoethanol, cystein and cystine. Theriogenology 53:761-771.

Funahashi H, Cantley TC, Stumpf TT, Terlouw SL, Day B. 1994. Use of low-salt culture medium with elevated oocyte glutathione levels and enhanced male pronuclear formation after in vitro fertilization. *Biol Reprod* 51: 633-639.

Furnus CC, de Matos DG, Moses DF. 1998. Cumulus expansions during in vitro maturation of bovine oocytes: Relationship level and its role on subsequent embryo development. *Mol Reprod Dev* 51: 76-83.

Gasparrini, B., Sayoud, H., Neglia, G., de Matos, D., Donnay, I., Zicarelli, L., 2003. Glutathione synthesis during in vitro maturation of buffalo (Bubalus bubalis) oocytes: effects of cysteamine on embryo development. Theriogenology 60:943-952.

Karja NWK, Kikuchi K, Fahruddin M, Ozawa M, Somfai T, Ohnuma K, Noguchi J, Kaneko H, Nagai T. 2006. Development to the blastocyst stage, the oxidative state, and the quality of early developmental stage of porcine embryos cultured in alteration of glucose concentrations in vitro under different oxygen tensions. *Reproductive Biology and Endocrinology* 4:54.

Kim I.H., A Van Langendockt, A. Van Soom, G. Van Roose, A.L.Casi, P.J.M. Hendriksen. and M. Bevers. 1999. Effect of exogenous glutathione on *in vitro* fertilization of bovine oocytes. *Theriogenology*. 52:537- 547.

Livingston T, Rich K, MacKenzie S, Godkin JD. 2009. Glutathione content and antioxidant enzyme expression of in vivo matured sheep oocytes. *Anim Reprod Sci* 116: 265-273.

Maedomari N, Kikuchi K, Ozawa M, Noguchi J, Kaneko H, Ohnuma K, Nakai M, Shino M, Nagai T, Kashiwazaki N. 2007. Cytoplasmic glutathione regulated by cumulus cells during porcine oocyte maturation affects fertilization and embryonic development in vitro. *Theriogenology* 67: 983-993.

Mayor P, Lopez-Bejar M, Rodriguez-Gonzalez E, Paramio MT. 2001. Effects of the addition of glutathione during maturation on in vitro fertilization of prepubertal goat oocytes. *Zygote* 9: 323-330.

Steel, R.G.D dan J.H. Torrie. 1995. Prinsip Dan Prosedur Statistika. Penterjemah Bambang Sumantri. Gramedia Pustaka, Jakarta.

Triwulanningsih, E., M. R. Toelihere, T. L. Yusuf, B. Purwantara dan K. Diwyanto. 2001a. Seleksi dan kapasitasi spermatozoa dengan metode percoll gradient untuk fertilisasi oosit dan produksi embrio in vitro pada Sapi (unpublished).

Triwulanningsih, E, P. Situmorang, I. G. Putu, T. Sugiarti, A. M. Lubis, D. A. Kusumaningrum, W. Caroline dan R. G Sianturi. 2002. Penggunaan Glutathione dalam Medium Fertilisasi Guna Meningkatkan Persentase Blastosis Embrio Sapi. *JITV Vol. 7. No. 2.*

Zuelke KA, Jeffay SC, Zucker RM, Perreault SD. 2003. Glutathione (GSH) concentrations vary with the cell cycle in maturing hamster oocytes, zygotes, and pre-implantation stage embryos. *Mol Reprod Dev* 64: 106-112.

# Blood Mineral Profiles of Simmental Breed Cattle with Different Feeding Systems and Reproduction Statues in Payakumbuh Region West Sumatra, Indonesia

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## Abstract

The present study aimed to evaluate mineral profiles of imported heavy breed cattlebased onmineral concentrations of blood plasma by considering feeding systems and reproduction statues. Samples of blood were collected from 30 female Simmentals which were divided in two different rearing and feeding systems (each 15 animals), including well-managed grazing pasturesat Padang Mangatas Breeding Center for Beef Cattle and simple-housed hand feeding bytraditional small-scale beef cattle farms distributed in Payakumbuh region.The experimental animals composed of three different reproduction statues of heifers, pregnant and non pregnant cows of each 5 animals.Blood samples were collected from the tail vein (v. coccygica). Blood plasma were then separated and analyzed for Ca, P, Mg, Fe, Mn,Cu, Se, and Zn. Data were statistically analyzed in completely randomizedfactorial design of 2x3x5. Results showed that mineral concentration of blood plasma were not significantly ($P>0.05$) affected by the feeding systems and animal reproduction statues.Considering the critical levels in the blood, some micro minerals of Mn, Se, and Cuwere found deficient in blood plasma.It implies that there is a need of dietary supplementation of micro minerals of Mn, Cu, and Seto ensure reproduction performances and to improve the production capacity of the imported breeding cattle.

Keywords: mineral nutrition, beef cattle nutrition

## 1.Introduction

Beef cattle production in Payakumbuh region of West Sumatra which is dominated by small-scale farm enterprises has shifted from local toexotic breeds, especially crossed-bred Simmental with higher body size and meat-carcass portion, resulting better growth performances than local beef breeds.However, their effort to increase their revenue was hampered by the expensive price of the exotic breed.In addition to limited population, productivity of Simmental was also constrained by low reproductive rate of cows due to various reproductive disorders, such as silent heat periods, delayed conception, poor fertilization, and postpartum infertility.

Low reproductive rates are presumably caused by mineral deficiency in feed.The livestockare almost entirely dependent on feeds consisting of fodder and crop residues. The feed come from diverse sources, like crop and plantation areas, river banks, rice fields, forest edges and roadsides.The livestock often suffer from malnutrition, while supplementation of ruminants with minerals or concentrated feed is not a common practice.Several incidents of mineral inadequacies in forages and soils have been reported, whichare principal causes of reproductive failure of imported dairy cattle in the tropical countries (Rukkwamsuk, 2011; Swai *et al*., 2005; Lyimo *et al*., 2004).

Table 1. Mineral concentration of blood plasma of cattle with feeding systems and reproduction statues

| Minerals | Farms | Reproduction status of cattle | | | Critical levels* |
|---|---|---|---|---|---|
| | | Heifer | Pregnant cows | Non-pregnant cows | |
| Ca, mg/100 ml | Small Farms | 10.26 (*0.43*) | 10.16 (*0.44*) | 10.56 (*1.01*) | <8.00 |
| | Grazing Pasture | 9.94 (*0.70*) | 11.70 (*0.40*) | 9.88 (*1.08*) | |
| P, mg/100 ml | Small Farms | 7.80 (*1.44*) | 5.04 (*0.91*) | 6.66 (*0.68*) | <4.50 |
| | Grazing Pasture | 7.54 (*0.71*) | 7.96 (*0.60*) | 9.04 (*1.85*) | |
| Mg, mg/100 ml | Small Farms | 2.56 (*0.16*) | 2.72 (*0.17*) | 2.18 (*0.34*) | <2.00 |
| | Grazing Pasture | 2.14 (*0.29*) | 2.88 (*0.25*) | 2.60 (*0.18*) | |
| Fe, ppm | Small Farms | 2.56 (*0.18*) | 2.27 (*0.19*) | 2.68 (*0.09*) | <1.10 |
| | Grazing Pasture | 2.80 (*0.07*) | 3.14 (*0.20*) | 2.80 (*0.16*) | |
| Zn, ppm | Small Farms | 2.60 (*0.44*) | 2.50 (*0.37*) | 2.26 (*0.28*) | <0.80 |
| | Grazing Pasture | 2.38 (*0.39*) | 2.59 (*0.43*) | 2.58 (*0.12*) | |

*Critical level suggested for cattle by McDowell (1997)

Proper mineral nutrition contributes to strong immune systems, reproductive performance and calf weight gain.The present research was aimed to evaluate mineral statues of Simmental breed cattleraised by small-scale farmers in comparison tothat raised in well-managed grazing pastures at Padang Mangatas Breeding Center for Beef Cattle based on mineral profiles of blood plasma.

## 2. Materials and Methods

Samples of blood were collected from 30 female Simmental breed cattle in two different locations of each 15 animals. The first was small-scale farms distributed in 50 Kota district and Payakumbuh city. The second was at the Padang Mangatas Breeding Center for Beef Cattle located at 50 Kota district. The experimental animals composed of heifers, pregnant and non-pregnant cows of each 5 animals.The non-pregnant cowsin the present studies mean that cows were not pregnant for long period of time after repeatedly artificial insemination services. Blood samples were collected from the tail vein (v. coccygica) using 10-mLdisposable syringes. The blood was then transferred to heparinized vials in order to avoid the clotting of blood. The blood samples were centrifuged at 3000 rpm for 20 minutes to separate blood plasma. The blood plasma was then preserved in refrigerated condition for determination of minerals.Mineral concentrations of Ca, Mg, P, Fe, Cu, Zn Se, and Mn in the plasma were determined by standard methods by using the atomic absorption spectrophotometer the Chemical Laboratory of National Veterinary Service Institute in Baso, Bukittinggi, West Sumatra, Indonesia.Data obtained in the present study were statistically analyzed by using variance analysis (ANOVA) in completely randomized factorial design of 2x3x5.Duncan's Multiple Range (DMRT) was applied to separate means. Differences were considered significant at $P<0.05$ (Steel*et al*., 1997).

## 3. Results and Discusion

Table 1 showed there was no significant different of mineral concentrations in blood plasma collected from different feeding systems and reproduction statues of cattle. Considering the critical levels the blood for Ca (8.0 mg/dL), P (4.5 mg/dL), and Mg (2.0 mg/dL) (McDowell, 1997), the results suggest that blood plasma Ca (9.88-11.70 mg/dL), P (7.54-9.04 mg/dL), and Mg (214-2.88 mg/dL) levels of were found in normal range limits in all animals. The adequate supplies of Ca, P, and Mg supported by Ca-P-Mg homeostasis (Lean *et al*., 2006) may explain

the rare incidences of reproductive disorders related with deficiencies of these macro minerals, such as dystocia, retention of placenta, prolapse of uterus, and embryonic death (Yasothai, 2014; Chaudhary and Singh, 2004; Kumar, 2003).

In term of trace elements, the average Fe (2.80-3.14 ppm) and Zn (2.38-2.59 ppm) concentrations in blood plasma (Table 1) were above critical level of 1.1 and 0.80 ppm, respectively (McDowell, 1997). Fe deficiency is extremely rare in grazing adult cattle, because Fe is ubiquitous in the environment, adequate content in soils and forages (McDowell and Artington, 2005; Shisia *et al*., 2013; Khan *et al*., 2007).However, Mn, Cu, and Se levels in blood plasma werenot detected in comparison to standard concentration of 0.005 mg/l (Table 1). Marginal deficiency of Cu, Zn, and Mn were also observed in grazing cattle and below the critical level in South Sulawesi of Indonesia (Prabowo *et al*., 1990). Khalil *et al*. (2015) found that some trace elements of Zn, Cu, and Se are located in marginal concentrations in wild forage in West Sumatra.

## Conclusion

It can be concluded that mineral concentrations in blood plasma of Simmental breed cattle in Payakumbuh region were not significantly affected by feeding systems and reproduction. The mineral Ca, P, Mg, Fe, and Zn were found in normal levels in the blood plasma, while several trace elements of Mn, Se, and Cu were deficient.It implies that the stocking rate of the grazing pasture could be increased and there is a need of dietary supplementation of micro minerals of Mn, Cu, Se, and Znto ensure reproduction performances and to improve the production capacity of the breeding center.

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## References

Chaudhary, S. and A. Singh, 2004. : Role of nutrition in reproduction: a review. Intas Polivet, 5: 229-234.

Khalil, M. N. Lestari, P. Sardilla and Hermon, 2015. The use of local mineral formulas as a feed block supplement for beef cattle fed on wild forages. Media Peternakan, 38(1):34-41.

Khan, Z.I., M. Ashraf, A. Hussain and L.R. McDowell, 2007. Seasonal variation of trace elements in a semiarid veld pasture. *Communications in soil science and plant analysis*, 37:1471-1484.

Kumar, S., 2003. Management of infertility due to mineral deficiency in dairy animals. In: Proceedings of ICAR summer school on "Advance diagnostic techniques and therapeutic approaches to metabolic and deficiency diseases in dairy animals". Held at IVRI, Izatnagar, UP (15th July to 4th Aug.). pp. 128-137.

Lean, I. J., P. J. DeGaris, D. M. McNeil and E. Block,2006. Hypocalcaemia in dairy cows: meta-analysis and dietary cation anion difference theory revisited. *J. Dairy Sci*., 89: 669-684.

Lyimo, Z.C., R. Nkya, L. Schoonman and F.J.C.M. van Eerdenburg, 2004. Post-partum reproductive performance of crossbred dairy cattle on smallholder farms in sub-humid coastal Tanzania. Trop. Anim. Health Prod. 36: 269 – 279.

McDowell, L.R. 1997. Minerals for Grazing Ruminants in Tropical Regions. 3rd ed. Bulletin. 81 p. Department of Animal Sciences, University of Florida, Gainesville

McDowell, L.R., and J.D. Arthington, 2005. Minerals for Grazing Ruminants in Tropical Regions. (4th ed.). Gainesville, FL, USA: University of Florida, IFAS.

Prabowo, A., L.R. McDowell, N.S. Wilkinson, C. Wilcox and J.H. Conrad, 1990. Mineral status comparisons between grazing cattle and water buffalo in South Sulawesi, Indonesia, Buffalo Journal,1 : 17-32.

Rukkwamsuk, T., 2011. Effect of nutrition on reproductive performance of post parturient dairy cows in the Tropics: A Review. Thai J. Vet. Med. Suppl. 41: 103-107

Shisia, K.S., V. Ngure, H. Nyambaka, and F.D.O. Oduor, 2013. Effect of pH and forage species on mineral concentrations in cattle breeds in major

grazing areas of Uasin Gishu County, Kenya. *Int.J.Curr.Microbiol.App.Sci.* 2(12): 247-254

Steel. R.G.D, J.H. Torrie, and J.H. Dicky. 1997. Principles and Procedures of Statistics: A Biometritrical Approach. 3rd Ed. McGraw-Hill Book Co. Inc., New York, USA.

Swai, E.S., M.J. Bryant, E.D. Karimuribo, N.P. French, N.H. Ogden, J.L. Fitzpatrick and D.M. Kambarage, 2005. A cross-sectional study of reproductive performance of smallholder dairy cows in coastal Tanzania. Trop Anim Health Prod. 37: 513-525.

Yasothai, Y., 2014. Importance of minerals on reproduction in dairy cattle. Int. J. of Sci., Environment and Technology, 3 (6): 2051 – 2057.

# Performance of Broiler Chickens Fed Turmeric and Zinc Mineral under Heat Stress

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## Abstract

This experiment was conducted to evaluate performance of broiler chickens fed turmeric and zinc minerals under heat stress. Eighty 14 day old broilers with 414,9 ±31 gram of average body weight were randomly allocated to five dietary treatments containingA: (without supplemented turmeric and zinc), B: (supplemented 0,2% turmeric and 40 ppm zinc), C: (supplemented 0,2% turmeric and 80 ppm zinc), D: (supplemented 0,4% turmeric and 40 ppm zinc) and E: (supplemented 0,4% turmeric and 80 ppm zinc). Each diet was fed to four replicates of 4 birds each. Heat stress was applied for 24 h (31-33ºC ) from 14 to 42 days. Feed intake and body weight were measured every week. Data collected were analyzed with Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) was futher used to the significant differences. The experiment resulted that supplementation of turmeric and zinc did not affect feed intake, body weight gain and feed conversion ratio on broiler. The feed intake of A, C,D and E was lower than B, meanwhile of C higher than A, D and E. Average of body weight gain of B was the highest compared with the other similarly with feed conversion ratio. It can be concluded that supplementation of turmeric 0,2% and zinc mineral 40 ppm could be agent of antistress, but not affected inperformance of heat stressed broilers.

Keywords :heat stress; performance; turmeric; zinc

## 1. Introduction

High environmental temperature may result in the accumulation of body heat that suffers from heat stress. Poultry like broilers is one of thehomeothermic species colud maintain their body temperature relatively constant by increasing water consumption and decrease of feed consumption. As a result growth rate and productivity of them will decrease. Feed consumption and body weight gain 5-8 weeks of age of broilers reared at temperature of 21$^{o}$C were169.9 g/d and 61.45 g/d respectively, significantly higher than for those reared at 34$^{o}$C with feed consumption 93.6 g/d and body weight gain 22.29 g/d [1]. However, feed conversion ratio increased from 2.76 at low temperature to 3.92 at high temperature.

Chronic heat stress may decreased metabolic oxidation capacity due to a self propagating scavenging system [2]. Nowday, many researches have been done about antioxidant properties of turmeric. Turmeric is a medical herb native to the Asian that has a considerable content of curcumin. Phenolic antioxidant is main antioxidant component in turmeric. Curcuminprevent lipids from oxidation [3]. In Asian countries, including Indonesia and China turmeric is used as a food additive, preservative and coloring agent. Turmeric has beneficial effects on many biological reactionsincludinganti inflammatory, antioxidant, anticardiogenic, antimutagenic, anticoagulant, antifertility, antidiabetic, antibacterial,antifungal, antipro-tozoal, antiviral, antifibrotic, antivenom, hypotensive and hypocholesteremic activities [4].

The performance parameters of broilers fed turmeric powder were improved

[5],[6]. Birds fed turmeric powder had lower feed intake and feed conversion ratio [7].Feed intake and body weight gain of 2 – 5 weeks old broiler at temperure 32$^o$ C (heater) was 1484 g and 692 g, significantly lower compared with temperature 22$^o$ C (AC) 2462 g and 1322 g respectively, meanwhile feed conversion ratio showed 2,14 in hot temperature and 1,86 in cold temperature[8]. Moreover Ref [9]stated that giving Turmeric significantly decreasing of feed consumption on broilers chicken in 2-7 weeksoldfrom 2505 g/bird (control) become 2410, 2455, 2430 and 2355 g/bird on level of turmeric 0.04, 0.08, 0.12 and 0.16 respectively. Decreasing of feed intake may caused by turmeric content of atsiri oil that had specials mell therefore impact to palatability of feed. Meanwhile suplementation 0,2% turmeric in broilers ration could be used as anti stress that showed in higher compared with control or the other doses of turmeric[10].

Zinc minerals are necessary for growth, structure and enzyme function also for body immunity that was investigated can use for solving not good effect from heat stress on birds [11]. Utilization of 40 ppm of zinc okside in feeding could be used for broilers under heat stress situation until 6 weeks of old[12]. The other finding showed that broilers need about 84 ppm of zinc sulphate until 21 days of old [13]. Therefore, the objective of this research was to find out the effect supplementation of turmeric and zinc mineral on performance of brolers under heat stress.

## 2. Material and Methods

Eighty 14 day old broilers with 414,9 ±31 gram of average body weight were randomized into 20 unit of cages. Feed (three times per day) and water were provided *ad libitum.* Ration was formulated base on nutritional requirement of chicks with 21% crude protein and 3000 kkal/kg of metabolism energy that consist of corn meal, rice meal, soybean meal, fish meal, coconout oil and top mix also supplementation of turmeric and zinc minerals according to each treatments (Tabel 1).

Dietary treatments containingA: (basal diet without supplemented turmeric and zinc), B: (basal diet supplemented 0,2% turmeric and 40 ppm zinc), C: (basal diet supplemented 0,2% turmeric and 80 ppm zinc), D: (basal diet supplemented 0,4% turmeric and 40 ppm zinc) and E: (basal diet supplemented 0,4% turmeric and 80 ppm zinc). A completely randomized was used with four replicates of 4 birds each. Heat stress was applied for 24 h (31-33ºC ) from 14 to 42 days by using 60 watt pijar lamp for each unit.

The measured variables cinsisted of feed intake, body weight gain and feed conversion ratio. Chicks weight and feed consumption were weekly recorded for each experimental unit. The feed conversion ratio was calculated (g feed: g gain). Data collected were analyzed with Analysis of Variance (ANOVA) and Duncant Multiple Range Test (DMRT) was futher used to test the significant differences [14].

## 3. Result and Discussion

Broilers fed turmeric and zinc mineral had similar feed intake, body weight gain and feed conversion ratio under heat stress (Table 2). The data revealed that supplementation of turmeric until 4 g/kg and zinc mineral until 80 ppm did not have influence on performance parameters. Similar findings about no alteration in performance parameters of broiler fed turmeric powder [15], [16][17].

Table 2 showed that average of feed intake in B was 3103.50 g/bird. It was higher than in A, D and E (3068.06; 3069.56 and 3077.19 g/bird respectively). It indicated that supplementation of turmeric and zinc impact to feed intake of broiler under heat stress condition. Feed consumption is esensial in economic analysis of broiler production. Utilization of 0.2% turmeric and 40 ppm zinc showed good impact for broiler performance.

Tabel 1. Compositition of diets containing turmeric and zinc for 14-42 days in broiler chickens under heat stress

| Ingredient | A | B | C | D | E |
|---|---|---|---|---|---|
| Corn | 54.7 | 54.496 | 54.492 | 54.296 | 54.292 |
| Rice brand | 5 | 5 | 5 | 5 | 5 |
| Fish meal | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 |
| Soybean meal | 18 | 18 | 18 | 18 | 18 |
| Top mix | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Coconout oil | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Turmeric powder | 0 | 0.2 | 0.2 | 0.4 | 0.4 |
| Zinc oksida | 0 | 0.004 | 0.008 | 0.004 | 0.008 |
| Total | 100 | 100 | 100 | 100 | 100 |
| Protein (%) | 21.064 | 21.065 | 21.064 | 21.065 | 21.065 |
| Fat (%) | 4.051 | 4.061 | 4.061 | 4.071 | 4.071 |
| Crude fiber (%) | 4.186 | 4.206 | 4.206 | 4.226 | 4.226 |
| Ca (%) | 1.393 | 1.138 | 1.138 | 1.138 | 1.378 |
| P (%) | 0.768 | 0.767 | 0.768 | 0.767 | 0.766 |
| ME (Kkal/kg) | 3050.70 | 3044.05 | 3043.92 | 3037.54 | 3037.41 |

Tabel 2. Performance of broiler chickens fed turmeric and zinc minerals under heat stress.

| Parameter | A | B | C | D | E |
|---|---|---|---|---|---|
| Feed intake (g/bird) | 3068.06 | 3103.50 | 3086.38 | 3069.56 | 3077.19 |
| Body weight gain (g) | 1295.44 | 1407.94 | 1306.75 | 1207.69 | 1224.69 |
| Feed conversion ratio | 2.38 | 2.21 | 2.37 | 2.54 | 2.52 |

[ab]Means with different superscripts within the same row differ significantly (P<0.05).

The highest of feed intake was found in B treatment and treatment A as control diet without supplement of turmeric and zinc showed the lowest of feed intake. These result indiated using turmeric and zinc could be anti stress agent in broilers.This study agree with the findings [10],utilization of 0.2% turmeric in broiler feeding could be anti strees agent. Moreover [12] reported zinc oxside as much as 40 ppm effectively applied on broilers under heat stress until 6 weeks old.

Average of body weight gain in broilers under stress heat amount 1207.69-1407,94 g/bird.The higher body weight gain was found in B, C compared to D, E and A, eventhough body weight gain in A was higher than D and E. this finding may caused by curcumin content in turmeric and zinc mineral that able to enhance the metabolism [18] followed by increasing ob body weight gain.

Turmeric and zinc mineral administration in broilers under heat stress was actually hoped to able to enhance the metabolism, followed could improve feed efeciency of broilers. However, this finding showed that FCR was not affected significantly (P>0.05) by turmeric and zinc oxide supplemented in ration (Tabel.2). These result was similar with Ref [19] but in contrast to Ref [29] who reported that turmeric improved feed efficiency of broiler at starter and finisher phase. Moreover, supplementation of turmeric powder did not effect FCR of broiler rabbit. The insignificant effect of turmeric and zinc on feed efficiency of broilers was associated with body weight gain and feed intake that were apparently not affected by turmeric administration.

## Conclusion

Turmeric and zinc seemed to able to be antioxidant agen for broiler under heat strees without effect to performance of broilers. That indicated that administration of 0.2% turmeric supplementation of turmeric 0,2% and zinc mineral 40 ppm could be agent of antistress, but not affected inperformance of heat stressed broilers.

## References


[1] Lu QJ Wen and H Zhang.2007. Effect of chronic heat exprosure on fat deposition and meat quality in two gnetic types of chicken. Poult Sci. 86:1059-1064.

[2] Azad MAK, Kikusato M, Maekawa T, Shirakawa H, Toyomizu M. 2010. Metabolic characteristics and oxidative damage to skeletal muscle in broiler chickens expose to chronic heat stress. Comp. Biochem. Physiol. 155(A):401-406.

[3] Sreejayan N, Rao MNA, Priyadarsini KI, Devasagayam TPA. 1997. Inhibition of radiation induced lipid peroxidation by curcumin. Int.J. Pharm. 151:127-130.

[4] Chattopadhyay I, Biswas K, Bandyopadhyay U, Banerjee RK. 2004. Turmeric and curcumin: Biological actions and medicinal applications. Curr. Sci. 87 (1) :44-53.

[5] Suvanated C, Kijparkorn S, Angkanaporn K. 2003, Effect of turmeric (*Curcuma longa linn*) as an antioxidant on immune status and growth performance of stressed broilers. The Chulalongkorn University Faculty of Veterrinary Science.

[6] Zeinali A, Kermanshahi H, Riasi A, Farhangfar H, Sarir H, Zaiaie H. 2009. Effect of Sodium Seleneteb and turmeric powder on growth performance, carcass quality and blood antioxidant metabolites of heat stressed broiler chickens. Anim Sci. Res. J. 19(2):69-85.

[7] Durrani FR, Ismail M, Sultan A, Suhail SM, Chand N, Durrani Z. 2006.Effect of different levels of fed added turmeric on the performance of broiler chicks. J. Agric. Biol. Sci. 1:9-11.

[8] Kusnadi E. 2004. The effect of pegagan (*Centellaasiatica*) on broilers response rearing at different environmental temperatures. J.AnimSci and Envi. 10: 10- 14.

[9] Bintang I.K, and A.G.Nataamijaya. 2005. Effect of addition turmeric powder *(Curcuma domesticaval)* in broilers ration. Proceeding of National Seminar of Husbandry Technology and Veteriner, Bogor, 12–13 September 2005, Bogor: 733 – 736.

[10] Kusnadi, E and A. Rachmat. 2010. Effect supplementation of turmeric *(Curcuma domesticaVal)* to changing of some of blood component and growth of broiler chickens under heat stress. Proceeding of National Seminar of Husbandry Technology and Veteriner. Recearch Centre of animal Development. Bogor.

[11] Sahin, K., and O.Kucuk. 2003. Zinc supplementation alleviates heat stress in laying Japanese quail. J. Nutr, 133:2808-2811.

[12] Lai, P.W., J.B.Liang, L.C.Hsia, T.C.Loh, and Y.W.Ho. 2010. Effects of varying dietary zinc levels and environmental temperatures on growth performanc, feathering score and feathering score and feather mineral concentrations of broiler chicks. Asian-Aust.J.Anim.Sci. 23 (7): 937 – 945.

[13] Huang,Y.L., L.Lu, X.G.Luo, and B.Liu. 2007. An optimal dietary zinc level of broiler chicks fed a corn-soybean meal diet. Poult.Sci. 86: 2582-2589.

[14] Stell RGD and JH Torrie. 1993. Principles and procedures of ststistic, 2nd Edn. Graw-hall.BookComp. New York.

[15] Gowda NKS, Ledoux DR, Rottinghaus GE, Bermuez AJ, ChenYC. 2008. Efficacy of turmeric (*Curcuma longa*) containing a known level of curcumin and a hydrate sodium calcium aluminosilicate to ameliorate the adverse effects of aflatoxin in broiler chicks. Poult Sci. 87:1125-1130.

[16] Mehala C, Moorthy M. 2008. Production performance of broilers fed *Aloe vera* and *Curcuma longa* (turmeric). Int J Poult Sci. 7:852-856.

[17] Yarru LP, Settivari RS, Gowda NKS, Antonio E, Ledoux DR, and Rottinghous GE. 2009. Effects of turmeric (*Curcuma longa*) on the expression on hepatic genes associated with biotransformation, antioxidant and immune system in broiler chicks fed aflatoxin. Poult Sci. 88:2620-2727.

[18] Al-Sutan, Gameel AA,2004. Histopathological changes in the livers of broiler chicken supplemented with turmeric (*Curcuma longa*). Int J Poult Sci. 3:333-336.

[19] Emadi M, Kermanshahi H. 2006. Effect of turmeric rhizome powder on immunity responses of broiler chickens. J Anim Vet Adv. 6:833-836.

[20] Durrani FR, Ismail M, Sultan A, Suhail SM, Chand N, Durrani Z. 2006. Effect of different levels of feed added turmeric (*Curcuma longa*) on the performance of broiler chick. J Agric and Biol Sci. 1:9-11.

# Utilization of Plant Titonia Flowers (Tithonia Diversifolia) in the Ration on the Performans of Broiler

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## Abstract

This study aims to look at the effect of the use of plant Titonia flowers (Tithonia diversifolia) in the ration on the performances of broiler. This study uses the Day Old Chick (DOC) CP 707 strain as much as 80 tails, flour plant Titonia flowers (TBT), commercial feed Vivo brand. The method used is an experimental method with Complete Random Design (RAL) using 4 treatments with the level of TBT in the ration of different namely A (0% TBT), B (5% TBT), C (10% TBT) and D (15% TBT) with 5 replications. Parameters measured were feed intake, body weight gain and feed conversion of broiler. Results showed that utilization of plant Titonia flowers (Tithonia diversivolia) in the ration was highly significant (P <0.01) in the feed intake, bodyweight gain and feed conversion of broiler. The best results in this study is in treatment B (5% starch plant flowers Tithonia diversifolia) ie 66.94 feed intake (g / bird / day), body weight gain 49.17 (g / head / day) and conversion ration of 1:52.

Keywords: feed intake, body weight gain, feed conversion, broiler

## 1. Introduction

Feed is the main need for basic living, growth, and reproduction for livestock production, but efforts to continuously feed supply more difficult necessitating alternative feed that is available in large quantities and continuously. The provision of high quality feed, especially for poultry still has obstacles, such as difficulties in obtaining feed ingredients, compete with human needs as well as feed prices are expensive and unstable ha linin because of several main raw materials are imported, such as corn, soybean meal, fish meal, flour meat, bone meal, and so forth.

One alternative to overcome the above problems is to use the plant in the form of interest Titonia (Tithonia diversifolia). Plants Titonia (Tithonia diversifolia) is a plant of the legume group of trees with the availability of abundant, easily accessible, of high nutritional value, it does not compete with human needs and still rarely used as animal feed, particularly poultry. Flowers Titonia contains high nutrient especially crude protein, wherein the nutritional content of interest Titonia the water content of 13.78%, crude protein 23:56%, crude fiber 22:13%, crude lipid 5:08%, Ca 1:14%, P 0:51%, ash 13.61% and ME 2566 kcal / kg (Integrated Laboratory Kopertis Region X, 2016).

Besides having a high protein, plant flowers Titonia has advantages not shared by other feed ingredients. Flowers Tithonia diversifolia pigment yellow color that is potentially containing ß-carotene which can be relied upon as a source of precursor of vitamin A in the digestive system of poultry especially in poultry laying hens and broilers, which will produce eggs that are high in vitamin A and improve the quality of the meat is low fat as well as antioxidant free-radical scavengers. Results of analysis obtained content of β-carotene contained in Titonia plants flower (Tithonia diversifolia) is 139.40

mg / kg (IPB Post Harvest Laboratory Analysis, 2015).

Based on the above background and potential of the flower Titonia (Tithonia diversifolia) in the form of nutritional value, are expected to boost productivity in broiler. Therefore, to investigate the effect of the use of flour Titonia plants flower (Tithonia diversifolia) in commercial ration on the performances of broiler.

## 2. Material and Methods

*2.1.Time and Place*

This study was conducted over 35 days at the start the second week of April to the second week of May 2016, at UPT Husbandry Faculty of Agriculture, University Islam Kuantan Singingi, Kuantan Singingi District.

*2.2. Tools and Materials*

The equipment used in this study a stable box of 20 units, 5 watt incandescent lamps as many as 20 pieces and one 20-watt incandescent bulb, where food and drinking water, scales, blenders and stationery.

The materials used in this study is a broiler strain CP 707 80 tail production PT. Charoen Phokphan Jaya Farm, a commercial ration Vivo brand as much as 62 160 kg ofCP 311 and CP 512 Vivo many as 89 477 kg and flour Tithonia diversifolia plant flowers as much as ± 14,000 kg.

*2.3. Research Methods*

This study was conducted experimen-tally using a completely randomized design (CRD) with 4 treatments and 5 replications. The treatment is distinguished by the amount of flour Flowers Award Titonia (TBT) in a commercial ration, with treatment that is: A = Giving TBT 0%, B = The award of TBT 5%, C = 10% Provision TBT and TBT Giving D = 15%.

*2.4. Parameters Measured*

a.Feed Intake

b.Body Weight Gain

c.Feed Conversion

*2.5. Data Analysis*

The data obtained in this study were analyzed by ANOVA (analysis of variance / ANOVA) based on completely randomized design (CRD). If obtained significantly different results, then continued by Duncan Multiple Range Test (DMRT) to determine differences between treatments (Steel and Torrie, 1995).

## 3. Result andDiscussion

*3.1. Feed Intake*

The average feed intake of broiler with flour of Tithonia difersifolia interest in the diet during the study are presented in Table 1.

Results of analysis of variance showed that administration of flour Tithonia interest in the ration provides a significant influence ($P < 0.01$) in the feed intake of broiler. Feed intake were highest in treatment B, namely 66.94 (g / head / day), while the lowest consumption in treatment D is 65.08 (g / head / day).

Based on the test results DMRT substitutions in treatment B ration significantly different with treatment A, D and C. A while treatment was not significantly different with treatment C and D. The differences were significant ($P < 0.05$) between treatments due administration of starch in the ration Tithonia flower an appropriate dose that increases appetite, one of the nutrients that can boost the consumption of β-carotene is an active substance that colors can enhance the body's immune system and improve feed intake and improve the digestibility in the body.

The real difference between the treatment and the treatments C and D can also be caused by high phytic acid compound in interest Tithonia diversifolia which may decrease feed intake of broiler. According Fasuyi et al., (2010) Compounds antinutrisi phytic acid is a substance that has the highest content on Tithonia diversifolia other

thananti-nutritive substances is as much as 79.1 mg/100g.

Table 1. Mean Consumption Broiler Rations During the study.

| Treatments | Rations Consumption (g/head/day) |
|---|---|
| A | 66.11b |
| B | 66.94a |
| C | 65.60bc |
| D | 65.08c |
| SE | 0.20 |

Description: superscript with different letters in the same column indicate significant differences between treatments (P <0.05).

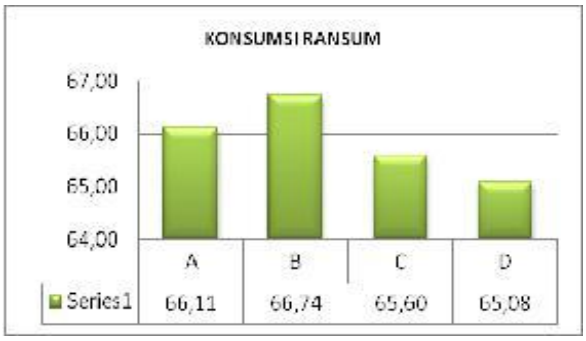


Fig. 1 Graph of the average feed consumption

Phytic acid compound has a complex molecular structure that can inhibit the absorption and binding protein and essential minerals, especially phosphorus thus decreasing the absorption of protein and minerals essential for livestock that consume (Davies, 1982). With the presence of these compounds, the use of Tithonia diversifolia be limited in the diet of livestock, especially poultry. Results of research Montesqrit et al., (2015), states that the use of flour Tithonia diversifolia on limited rations pitalah duck which can only reach the level of 10% if more than that level can cause a decrease in feed intake.

From the Fig. 1 above it can be seen feed intake of the highest order are in treatment B, namely 66.94 (g/head/day), treatment A is 66.11 (g/head/day), C treatment is 66.60 (g/head/day) and D treatment that is 65.08 (g/head/day). The graph shows a downward trend broiler feed intake with an increased level of interest Tithonia diversifolia starch in the ration. This is caused by differences in crude fiber content of the flour along with the addition Thitonia interest in the ration.

High fiber in the interest Tithonia diversifolia can decrease feed intake of broiler because a higher level of use Tithonia flour will increase crude fiber content of the feed so that raw fiber consumption also increased. According to the results of laboratory analysis of Integrated Kopertis Region X (2016) Tithonia diversifolia flower containing coarse fiber of 22:13%. In this study, the addition of flour interest level Tithonia diversifolia granting 5% in the ration still tolerable than that of 10% and 15%. This is because the content of crude fiber ration treatment B is equal to 5.85%, while treatment C is 6.71% and treatment D 7:56%. According to BSN (2006) limits the content of crude fiber in the ration of broiler maximum 6%.

According Wahju (2004) factors affecting feed intake is the energy content and palabilitas rations, the type of chicken, weight and crude fiber rations. Bahri, et al (2008) suggested that high fiber is not only difficult to digest but also caused some food substance shipped out in excreta. Difficulty digesting food ingredient that causes broiler stop consuming feed for capacity cache has been fulfilled. Increased use of coarse fibers potentially also reduce feed consumption due to the higher crude fiber causes the feed is bulky so that birds can not consume food in sufficient quantities as a result of the limited capacity of the cache (Samli et al., 2006).

Wahju (2004) adds that the main factors affecting feed intake is the metabolic energy content in the feed as well as high fiber, can not be utilized by birds because the birds do not have the enzymes to digest fiber. Feed quality in formulated feed should be noted

that feed that has been formulated it is able to be obtained livestock concernedand the amount of nutrients that are needed can be met because each animal has a limitation in consuming feed (Siregar, 1980).

The results showed that the use of Tithonia diversifolia flower flour as much as 5% in the ration gives the best result. The results of the study the average feed consumption of broiler chickens aged 5 weeks ranged between 65.08-66.94 (g / head / day). The total average feed intake results of the study ranged from 2279-2335 (gr / tail) is lower than the results of Guidance Service PT. Charoen Pokphand (2006), namely a total feed intake of broiler age of 5 weeks of 2912 (g / head). This difference may be caused by the amount of feed intake, dietary protein, the environment, climate, temperature and cage management of broiler.

*3.2. Body Weight Gain*

Body weight gain of broilers per cow per day can be seen in Table 2. Based on the data in Table 2 it can be seen that the average body weight gain of broilers from the order of highest to lowest is B (5% starch interest Tithonia) is 47.17 (g/head/day), A is 46.29 (g/head/day), C is 45.40 (g/head/day) and D is 44.41 (g/head/day). Based on the results of statistical analysis showed that the Tithonia difersifolia flour interest in the ration provides a significant influence (P <0.01) in the weight gain of broilers.

Table 2. Mean Body Weight Added Broiler During the study.

| Treatments | Body Weight Gain (g/head/day) |
|---|---|
| A | 46.29b |
| B | 49.17a |
| C | 45.40c |
| D | 44.41d |
| SE | 0.26 |

Description: superscript with different letters in the same column indicate significant differences between treatments (P <0.05).

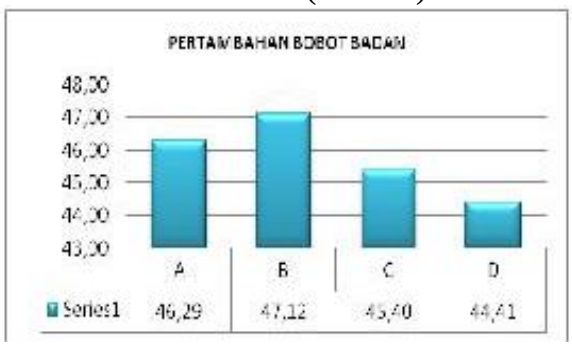


Fig. 2 Graph of the average body weight gain

Based on Duncan's Multiple Range Test can know the difference between the treatment and significantly different (P <0.05) with treatment A, C and D. This is because the level of feed consumption in treatment B is also high, so the level of feed intake is directly proportional to body weight gain. Kartadisastra (1994), states that the weight loss was determined by the number of chickens will feed consumption. The bigger the chicken body weight, the more the number of feed consumption. High feed intake causes the amount of nutrients that enter the body of cattle used to meet basic living and for the growth of broiler, which are characterized by a high body weight gain.

According Ichwan (2003) that in general the weight gain will be affected by the amount of uneaten feed intake and nutrient content contained in the feed. This is supported also by the opinion Abidin (2002) that, factors that affect weight gain is the feed consumption.

In the graph above can be seen weight gain is highest in treatment B, namely 47.17 (g/head/day) and the lowest is D treatment that is 44.41 (g/head/day). The results showed that the use of Tithonia diversifolia flower flour as much as 5% in the ration gives the best result.

The results of the study the average weight gain of broilers age of 5 weeks ranged between 44.41 - 46.29 (g/head/day). This

result is lower than the Guidelines Service PT. Charoen Pokphand (2006) is a weight gain of broilers ranged between 76.40-83.10 (g/head). The low yield may be caused by the amount of feed intake, feed nutrient content, environment, climate, temperature and maintenance management and housing of broiler.

*3.3. Feed Conversion*

The mean value of the feed conversion ration per cow per day each - each treatment can be seen in Table 3.

Based on the results of statistical analysis showed that the Tithonia difersifolia flour in ration provides highly significant effect (P <0.01) in the feed conversion. The average feed conversion of broiler is 1.54 and ranged from 1.57 until 1.52. Based on Duncan's Multiple Range Test B treatment were significantly different (P <0.05) to the treatments C and D but not significantly different with treatment A.

From the chart above can be seen at the lowest feed conversion treatment B is 1:42 and the highest conversion on D is 1:47. The lower the number the better the feed conversion of broiler ability to optimize feed into meat. According Anggorodi (1995), the high and low feed conversion is determined by the balance between energy metabolism of nutrients, especially protein and amino acids. Card and Neisheim (1972)states that the value demonstrated high feed conversion ration the amount needed to raise the body weight increases and the lower feed efficiency.

Kartasudjana (2005) states efficient or not a feed given to broiler can be seen from the figures feed conversion. Samsiar (2004), adding the smaller the number the ratio between the amount of feed intake with the UN means the better the feed conversion rate. Both the poor feed conversion is influenced by various factors, including the quality of feed, animal health and procedures for the provision of rations.

Table 3. Mean feed conversion broiler rations during the study.

| Treatments | Feed Conversion |
|---|---|
| A | 1.53bc |
| B | 1.52c |
| C | 1.55ab |
| D | 1.57a |
| SE | 0.009 |

Description: superscript with different letters in the same column indicate significant differences between treatments (P <0.05).

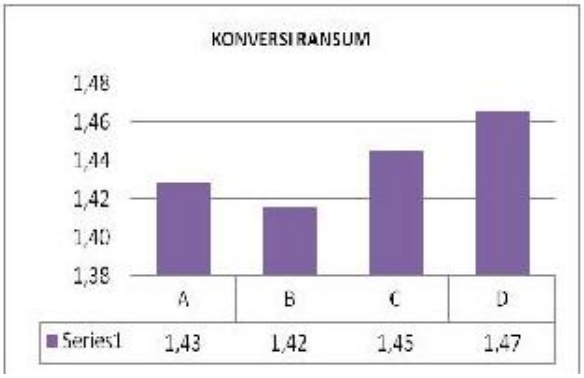


Fig. 3. Graph average feed conversion

The results showed that the use of Tithonia diversifolia flour as much as 5% in the ration gives the best result. The average feed conversion of broiler results of the study ranged from 1:52 to 1:57. These results are consistent with the standards of Charoen Pokphand Indonesia (2006), ie feed conversion CP 707 broiler aged 5 weeks at 1:56. But this result is not much different from the results Rifqi (2008) ranging from 1:42 to 1:52. Lacy and Vest (2004) stated that the main factors affecting feed conversion is genetic, ventilation, sanitation, food-quality, type of feed, as well as management and feeding.

**Conclusion**

From the results of the study concluded that giving Titonia flour (Tithonia difersifolia)

in the ration was highly significant (P <0.01) in the feed intake, body weight gain and feed conversion of broiler. The best treatment in this study is in treatment B (5% interest flour Tithonia difersifolia) ie 66.94 feed intake (g/ bird/day), body weight gain 47.17 (g/head/day) and conversion ration of 1:42.

## References


[1] Abidin, Z. 2002. Fattening Beef Cattle. Jakarta: PT. Agro Media Pustaka.

[2] Anggorodi, R. 1995. Livestock Food Science General. Jakarta: PT. Gramedia.

[3] Badan Standarisasi Nasional Indonesia. 2006. Indonesian National Standard :. Standard quality requirements for rations quail adult spawners (Quail Layer). http://cjfeed.co.id. [21 Maret 2016].

[4] Bahri, S dan Rusdi. 2008. Evaluation of Metabolic Energy Feed Local On Laying Chickens.http://jurnal.untad.ac.id/jurnal.index.php /AGROLAND/article/view/l6 63/135. Accessed on February 14, 2016.

[5] Card, L. E. dan M. C. Nesheim. 1972. Poultry Production. 11 Ed. Lea Dan Febinger, Philadelphia.

[6] Charoen Pokphand Indonesia. 2006. Manual Broiler Manajemen Lohmann. Jakarta: PT. Charoen Pokhpand Indonesia.

[7] Charoen Pokphand Indonesia. 2006. Manual Broiler Manajemen CP 707. Jakarta: Charoen Pokhpand Indonesia.

[8] Davies, N. T. 1982. Effects of phytic acid on mineral availability. In Dietary Fiber in Health and Disease. Vahoung, G. V. and Kritchevsky, D., Eds., New York: Plenum Press.

[9] Fasuyi A. O, Dairo F. A. S, Ibitayo F. J. 2010. Ensiling wild suflower (Tithonia diversifolia) leaves with sugar cane molases. Livest. Res Rural dev. 22:42.

[10] Ichwan. 2003. Make Broiler Feed. Print I. Jakarta: PT Agromedia Pustaka Utama.

[11] Kartadisastra, H. R. 1994. Chicken Feed Management. Yogyakarta: Kanisius.

[12] Kartasudjana, R. 2005. Poultry Management. Fakultas Peternakan. Bandung:Universitas Padjajaran.

[13] Lacy dan L.R. Vest. 2004. Improving feed conversion in ayam pedaging : A. guide for growers. http://www.ces. uga.edu/pubcd.c:793-w.html. [4 Februari 2016].

[14] Montesqrit, Dwi Ananta dan Yulia Mimi. 2015. Influence of Moon Flower Bushes (Tithonia diversifolia) Performance Against Pitalah Ducks. National Seminar on Food Security and Sustainable Agriculture. Agricultural Polytechnic. Payakumbuh 7 oktober 2015 (In Publish).

[15] Rifqi, A. 2008. Effect of Feed Additive "RI.I" and Type Feed Different Form Of Broiler. [Skripsi]. Program Science and Nutrition Faculty of Animal Husbandry, Bogor Agricultural University, Bogor.

[16] Samli H.E., N. Senkpylu, H. Akyurek, A and A. Agma. 2006. Using Rice Brain in Layer Diets. J. Cent. Eur. Agric. 7 (1): 135-140

[17] Samsiar, N. 2004. Stress food on the growth of the compensation and performance of broiler. Skripsi. Padang: Faculty of Animal Husbandry Andalas University.

[18] Santoso. Y. Fenita dan Kususiyah. 2009. Use of Water Extract Leaves katuk as a feed additive for Producing Meat Designers Efficient. Reports Featured Research Universities. Bengkulu: Bengkulu University.

[19] Siregar, A. P., M. C. Sarbani dan P. Suroparwiro. 1980. Mechanical raising broilers in Indonesia. Print I. Jakarta: Margie Group.

[20] Steel, R. G. D. dan J. H. Torrie. 1995. Principles and Procedures of Statistics. Interpreter: Sumantri, B. Jakarta: PT. Gramedia Pustaka Utama.

[21] Wahju, J. 2004. Poultry Nutrition Sciences. Yogyakarta: Gadjah Mada University Press.

# Analysis of Factors at That Influence Palm Oil Farmers Personal Income Trough Buffalo's Breeding

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## Abstract

Palm oil plays the main role in comercial agricultural plantation. It increases the income for both farmer and local people:in the other side it also producers waste including stems, pallm leaffs, and copra residue. Copra residue is classified as industrial wase taht can be consumed by livestocks, adding copra residue as a fodder option will help the breeder in supplying green woof. This research is conducted for the following purposes 1) To analyze the factors that influence palm oil, farmer's personal income.2) To describe the benefit of buffalo breeding in increasing farmers personal income. The sampel are taken from palm oil farme's who have buffalo breeding in Lubuk Basung. At least 36 farmers are indicated to run both palm oil plantation and buffalo breeding at the same time. The result of the analysis shows that independent variabels the vast of plantation (x1), palm oil production (X2), plant life spam (X3), the scale of life stocks ownerships (X4), labor (X5), fertilizer expenses (X6), and prices (X7), those variabels have significant role in determining farmer's personal in come. In partial, the vast of plantation and lifestock ownership have no significant influence.Toward farmer's personal income.coevesien determinate value ($R^2$) is 0,99 that shows independent variables contribution toward. Farmer's income around 99 % while other 1 % caused by other factors taht will not be described in this research. The it also indicates that lifestock value on new farmers is 10,5 %.

Keywords : Regretion linear, palm oil farmers, farmer's personal income, buffalo

## 1. Introduction

Palm Oil as a commodity crop that has an important role in economic activities in Indonesia. Besides as one of the country's foreign exchange, oil palm is also labor-intensive (labor intensive) to absorb the labor force (Oil Palm Cultivation Series). Based on the status Oil and gas, oil palm plantations are divided into folk

(PR), large country estates (PBN), and private estates (PBS). Of the three types of the concession, PBS 50.08% controlled acreage of oil palm Indonesia, PR36,71%, while PBN is only 13.20%. Increased acreage of the largest palm oil occurred in the period before the crisis (1980-1997 year) with a growth rate of 14.68% per year. Significant growth occurred in the area of oil palm PR and PBS respectively by 46.85% per annum and 19.79% per year, while PBN acreage increased by only 6.09% per year. In 2013 the total area of oil palm plantations has reached 10.01 million hectares. (Indarti, 2014)

Buffaloes have been developed / maintained by the community as a livelihood in the business scale is still relatively small, for the purpose of meat, leather and labor Increased role of buffaloes in support of the national need of meat is expected to support the adequacy of meat, because it can provide

an alternative a protein source provider meat producer. Obstacles faced by farmers is in the dry season forage difficult to get even a farmer will sell their livestock for forage difficulties. Efforts to increase livestock production is not enough just to give a natural grass are inferior, but it should be the availability of both the quantity and quality of forage.

One of the feed material of considerable potential and available is a byproduct of oil palm plantations (leaves and palm fronds). According to research conducted by (Parulian, 2009), the response of cattle to administration of palm leaves with the addition of the composition of the mixture of urea, salts, minerals, bran and molasses, livestock merspon well and within 14 days occurred weight gain weight on cattle, with thus it can be seen that the use of waste palm leaves beneficial for farm animals. This provides an opportunity for farmers in utilizing unused waste, which is beneficial from the plantation and agriculture as an alternative feed source (Asmandani & Susilo, 2013)

The problems that exist now, whether the development of oil palm plantation area is so rapid has been used for buffaloes namely in terms of the utilization of waste next to palm groves, which can add increasing population and livestock production. On that basis, this study aimed to find out; 1) factors affecting the income of farmers of oil palm plantations, 2) contribution of buffaloes on the income of farmers of oil palm plantations.

## 2. Materials and Methods

This research was conducted in the district Lubuk Basung Agam West Sumatra Province from March to September 2016. The object of the research into oil palm plantations are farmers who own buffaloes. The research sample was taken purposively as many as 36 farmers. The technique of collecting primary data through direct observation and interviews with a guide questionnaire and secondary data obtained from research institutions associated with them: Department of Animal Husbandry and services Plantation. Data were analyzed with multiple regression.

## 3. Results and Discussion

### 3.1 Characteristics of respondents

Age farmers gardens dominated by age 31- 50 years with the 61%. Age is an important population characteristics, because the age structure may affect the behavior of the socio-economic.

From the research results vary farmers aged between 27-71 years. Age farmers will affect physical ability and response to things that are new in running his farm.

Table 1. Characteristics of Respondents Age

| **Age respondents** | **Number** | **Persetase respondents (%)** |
|---|---|---|
| 15-30 years | 12 | 33 |
| 31-50 years | 22 | 61 |
| > 50 years | 2 | 6 |

*Source: From the research, 2016*

Table 2. Education level of respondents

| Education Level | Number of respondents | Percentage (%) |
|---|---|---|
| 1. Not completed primary school | 6 | 17 |
| 2. complete primary school | 19 | 53 |
| 3. junior high school graduation | 4 | 11 |
| 4. graduating from high school | 6 | 17 |
| 5. Universities | 1 | 2 |

*Source: Research data, 2016*

Table 3. The oil palm estates farmers

| Extensive palm gardens premise | Number of respondents | Percentage (%) |
|---|---|---|
| < 1 Ha | 3 | 8 |
| 1 – 2 Ha | 18 | 50 |
| > 3 Ha | 15 | 42 |

*Source: Research data in 2016*

One indicator of the quality of the population is education, education can be measured by formal education I've ever taken. Education for farmers have influence in the adoption of technology and farm management. Education is considered as a means to improve the knowledge of new agricultural technology, further education inculcate attitudes towards the use of modern farming practices. The results of the study illustrate, the share of education that most farmers just tannic elementary education level by 53%.

As an economic resource for rural communities, especially farmers, agricultural land is crucial production and farmers' household income (Mardikanto, 2010). Table 3 shows the data area of land owned. Variations of land owned by farmers starting from 0.5 to 15 hectares, and 50% have a land area of 1-2 hectares.

Table 4. Ownership buffaloes

| Number of animals | Number of respondents | Percentage (%) |
|---|---|---|
| 1 – 2 tails | 12 | 33 |
| 3 – 5 tails | 18 | 50 |
| ≥ 5 tails | 6 | 17 |

*Source: Research data in 2016*

Table 5. Income respondents from oil palm plantation and buffaloes (Rp / year)

| Sources of income | (Rp / year | Percentage (%) |
|---|---|---|
| 1. Palm oil plantation | 29.519.555 | 10,5 |
| 2.Buffaloes | 3.127.777 | |

*Source: Research Data*

Table 6. Analysis of variance estimators pendapatan[b] and outcome parameters

| Model | Df | F h | Sig. |
|---|---|---|---|
| Regression | 7 | 1338,528 | ,000[b] |
| Residual | 28 | | |
| Total | 35 | | |

*Source: Research Data,2016*

Total ownership of buffaloes can determine the amount of additional revenue obtained by farmers and also can be used as one of the socio-economic indicators. Farmers who have cattle under 5 animals as much as 83%, this is because farming is not the main job but as a sideline. Variations livestock ownership by farmers ranges from 1 to 9 tails.

Source: Research data in 2016 Palm Plantation Farmer Income Revenue can be used as a measure of household welfare. Sources of income of rural households can be divided into two, namely; income from farm and off-farm income from. Table 5 showed him the average income of oil palm plantations is Rp 29,519,555 per year, revenue from buffaloes Rp 3,127,777 per year. Total revenue is already in excess of the minimum wage of West Sumatra Rp 1,800,725, -

### 3.2. Independent Variable on Palm Plantation Farmer Income

To examine the factors that affect the income of farmers of oil palm plantations used multiple linear regression analysis, which is becoming independent variable / independent is the land area (X1), age of the plant (X2), production of oil palm plantations (X3), the scale of livestock ownership (X4) , labor (X5), Cost of fertilizer (X6) and the oil Price (X7), while being tied variavel / dependent is a palm oil plantation farmers' income (Y).

The results of the testing that affect the income of farmers of oil palm plantations can be seen in Table 6 and

Based on the results obtained persamaam regression analysis as follows:

Y = -2186950 -246795 X1+220192,89 X2+17291,27X3+182697,05X4+358474,67X5-3,51X6+0,57X7

Information:
Y = income of farmers of oil palm plantations
X1 = area of land
X2 = Age of plants

X3 = Production
X4 = Scale livestock ownership
X5 = Workforce
X6 = Cost of fertilizer
X7 = Price

Results of data analysis obtained jointly independent variable that consists of land area (X1), age of the plant (X2), the production of oil (X3), the scale of livestock ownership (X4), labor (X5), the cost of fertilizer (X6) and oil prices (X7) have a significant effect on the incomes of farmers partial palm plantations sawit. spacious garden and fertilizer costs do not have a significant effect on the income of farmers. The coefficient of determination ($R^2$) of 0.99 menunujukkan the contribution of all independent variables were able to explain the relationship of the pendapatn by 99% while the remaining 1% due to other factors that can not be explained in this study. (Goenadi & Sc, 2005)

Interpretation of the above models on cattle ownership scale independent variable (X4) explains, if the independent variable scale livestock ownership (X4) increased 1 ST, there will be increase in income (Y) of Rp 184 697. Donations opinions of buffaloes was a kid, this is because the scale of the ownership of small livestock. only reached 10.5%. The higher scale businesses run and owned cattle, the greater the acceptance will be accepted and can also suppress the production costs incurred. (Krishna and Mansur cit Rusdiana, S, 2011)

## Conclusion

The regression results there is a relationship between land use, age of the plant, production, livestock scale land ownership, labor, cost of fertilizer prices and farmers' income terhadapa oil palm plantations with dterminasi coefficient (R) of 0.99 was obtained. Contributions buffaloes to farmers' income by 10.5%.

## Acknowledgments

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## References

Asmandani, D. A., & Susilo, B. (2013). Waste Treatment Leaves Oil Palm (Elaeis guineensis Jacq) And Dregs of cassava as an Alternative Feed Supplement For Ruminant Processing of Waste Palm Leaf (Elaeis guineensis Jacq) and Cassava pulp As an Alternative Aditional Feed For Livestock (ruminants), 1 (1), 29 -34.

Goenadi, I. D. H., & Sc, M. (2005). Prospects and direction of development of oil palm agribusiness in Indonesia.

Hafiff, Bariot. Rr.Ernawati, and Y. P. (n.d.). The potential for increasing productivity of oil palm in Lampung province. Lintri, 20 (2), 6.

Indarti, D. (2014). Outlook palm oil commodity. (Ms. Ir. Dewa Ngakan Cakrabawa, MM. Dr. Ir. Leli Nuryati, Ed.). Center for Data and Information System for Agricultural Secretariat General - Ministry of Agriculture.

Mardikanto. (2010). Measure Adoption Rate With Three benchmarks. Retrievedfrom (http://www.google.cm

Rusdiana, S, I.-G. A. P. M. and C. T. (2011). (Farm Business Income in Buffalo in the District of Gunung Sindur Bogor) (pp. 152-158).

# Nutritional Value of Persimmon Yoghurt (*Dyospyros kaki*) as Healthy Soft Drink to Make Healthy and Fitness: An Analysis

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## Abstract

Innovation research has been conducted to make yoghurt used enzyme from Persimmon fruit. Material was used are Persimmon fruit with the grade of maturity 70-80% and sweetened condensed milk. Manufacture of fermentation done with 10 kg Persimmon fruit washed and stems discarded. So be sliced and put in to the sterile container and closed thightly, air tight not expose to direct sun light as long as 2 weeks. Processed an aerobic auto fermentation will happen. Then take the liquid as starter. The process of making yoghurt 10 ml sweetened condensed milk to glass 250 ml, and add starter 10 ml then stir homogeneus. Yoghurt Persimmon ready to drink. The result of nutritional value Persimmon Yoghurt e.g water 92,93%, vitamin C 11,85 mg/100 gram, tannin 881,2 mg/liter, vitamin B1 : 4864,23 mg/kg, vitamin B2 : 85,87 mg/kg, carbohydrate 5,73%, protein 9,24%, fat 0,85%, alcohol 0%, mineral 0,48% (e.g Al 0 mg/l, Cu 0,48 mg/l, Ca 154,25 mg/l, Fe 3,18 mg/l, Mg 149,87 mg/l, Zn 1,75 mg/l, Ni 0,24 mg/l, Cd 0 mg/l, Pb 1,37 mg/l, Cr 0,25 mg/l). Total bacteria 5,0 x 10 CFU/ml. *Salmonella shigella* negative and *Escherichia coli* negative. Antioxidant to counteract to free radical can prevented cancer, vitamin C increase immune respond again virus, bacteria, mold and dangerous substance and increase white blood production. Cu and Fe as a raw material red blood cell production and increase blood circulation, vitamin B complex as an enzyme will increase body metabolism. Tannin can decrease level of cholesterol and hypertension, treat diabetes mellitus, heart disease, cough, ashma and indigestion. The result this analysis will be concluded that Persimmon Yoghurt very good to maintenance healthy and fitness our body naturally as healthy soft drink.

Keywords : yoghurt, persimmon, nutritional value, healthy, naturally

## 1. Introduction

Persimmon fruit is a golden yellow, round or oval, flavorful, smooth textured delicacy from far East Asian origin. Its sweet, delicious flesh is packed with several health promoting nutrients such as vitamins, minerals, and anti-oxidants vital for optimum health. Botanically, persimmons belong to the family of *Ebenaceae*, in the genus: *Diospyros*. This delicate fruit is native to China. From China, spread to Korean Peninsula and Japan very long time ago, and later was introduced to California during the middle of the 19th century as in [1], [2], [19].

Condensed milk is cow's milk from which water has been removed. It is most often found in the form of sweetened condensed milk (SCM), with sugar added, and the two terms "condensed milk" and "sweetened condensed milk" are often used synonymously today. Sweetened condensed milk is a very thick, sweet product which when canned can last for years without refrigeration if not opened. Condensed milk is used in numerous dessert dishes in many countries [2].

Extract Persimmon and sweetened condensed milk can make yoghurt as a

delicious soft drink. Nutritional value this yoghurt is good to make our healthy and fitness. Persimmon fruit is moderately high in calories (provides 70 calories/100 g) but very low in fats. Its smooth textured flesh is a very good source of dietary fiber. 100 g of fresh fruit holds 3.6 g or 9.5% of recommended daily intake of soluble and insoluble fiber. Persimmons contain health benefiting flavonoid poly-phenolic anti-oxidants such as an important anti-tumor compound anti-infective, anti-inflammatory and anti-hemorrhagic (prevents bleeding from small blood vessels) properties [1], [3], [9].

Some of other anti-oxidant compounds found abundantly in this fruit are vitamin-A, *beta-carotene, lycopene, lutein, zea-xanthin and cryptoxanthin.* Together, these compounds work as protective scavengers against oxygen-derived free radicals and reactive oxygen species (ROS) that play a role in aging and various disease processes.*Zea-xanthin*, an important dietary carotenoid, is selectively absorbed into the retinal macula lutea in the eyes where it thought to provide antioxidant and protective light-filtering functions. [1].

Persimmons are also a very good source of vitamin-C, another powerful antioxidant Regular consumption of foods rich in vitamin C helps the body develop resistance against infectious agents and scavenge harmful, pro-inflammatory free radicals and anti cancer [1], [14], [15], [16], [17], [18], [19].It is good in many valuable B-complex vitamins such as folic acid, pyridoxine (vitamin B-6), thiamin...etc. [1].

Based on very important Porsimmon to our health so this research design to make Persimmon more delicious to consume with combine by sweetened condensed milk as fresh yoghurt and analysis nutritional value of auto fermentation yoghurt Persimmon as a starter.

## 2. Material and Methods

*2.1.Material*

- Persimmon fruit with the grade of maturity 70-80% 10 kg
- Sweetened condensed milk
- Vacuum sterile container
- Knife
- Glass
- Spoon

*2.2. Methods*

2.2.1.Making process microbial fermentation

-10 kg Persimmon fruit stemmed and Clean wash
- slice fruit
- put in sterile container air-tight, not expose to sun light
- save 2 weeks
- take the liquid as a starter

2.2.2. Making process of fruity yoghurt

- Put in 10 ml sweetened condensed milk in the glass
- Add starter 10 ml, mix homogenous
- Add water suit one's taste (cold, natural, hot)

## 3. Result and Discussion

### 3.1. Bacterial Analysis

Yoghurt production in this research test on bacterial contamination. The result of bacterial contamination are negative *Salmonella shigella* and negative *Escherichia coli*. Total bacterial 5x10 CFU/ml, very small and not dangerous. This fact indicate that operational procedure in this research enough sterile and hygienic.

*3.1.1. Salmonella shigella*

*Salmonella* are a group of bacteria that can be divided into typhoid *Salmonella* (*Salmonella typhi*) which causes typhoid fever and non-typhoid *Salmonella*. The latter is commonly known for causing salmonellosis which is a type of foodborne intestinal infection contracted after eating food contaminated with the *Salmonella* bacteria. These types of intestinal infections are more likely in children or the elderly. *Shigella* are a family of bacteria that cause an infectious

Fig. 1. (a)10 kg Persimmon; (b) slice fruit (c) sterile container

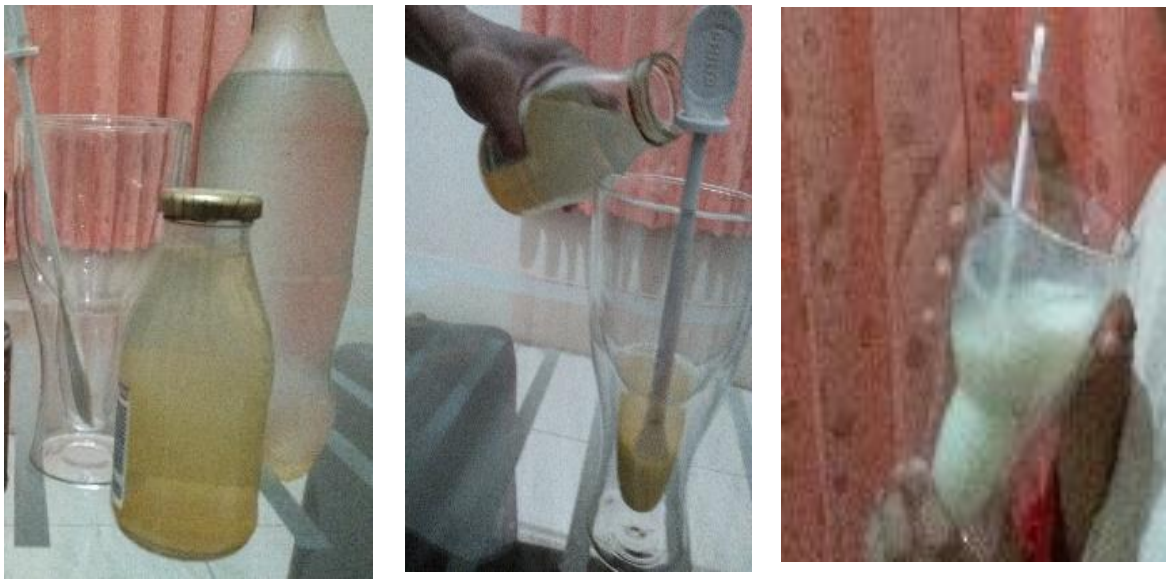

Fig. 2. (a) Starter, Milk and Water (b) Mix Starter, Milk dan Water (c) Yoghurt ready

intestinal disease known as shigellosis. It is mainly transmitted through contact with an infected person and contaminated food and water. Shigellosis can occur in any age group but is more commonly seen in children. It is one of the common causes of outbreaks of bacillary dysentery [3].

*3.1.2. Escherichia coli*

*Escherichia coli* (abbreviated as *E. coli*) are bacteria found in the environment, foods, and intestines of people and animals. *E. coli* are a large and diverse group of bacteria. Although most strains of *E. coli* are harmless, others can make you sick. Some kinds of *E. coli* can cause diarrhea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses [4].

**3.2. Proximate Analysis**

3.2.1. Alcohol

Liquid of Persimmon this research is zero alcohol. This is indicate yoghurt Persimmon as halal soft drink for moeslim as majority Indonesian people.

Table 1. Analysis Bacterial of Persimmon Liquid Auto Fermentation

| Parameter | The Result of Analysis | Unit of Measure | Methods |
|---|---|---|---|
| Total bacteria | $5 \times 10^1$ | Cfu/ml | - |
| *Salmonella shigella* | Negative | - | Qualitative |
| *Escherichia coli* | Negative | - | Qualitative |

Table 2. Analysis Proximate Nutritional Value of Persimmon Liquid Auto fermentation

| Parameter | The Result of Analysis | Unit of Measure | Methods |
|---|---|---|---|
| Water | 92.93 | % | SNI-01-2981-1992 |
| Alcohol | 0 | % | - |
| Carbohydrate | 5.73 | % | By difference |
| Fat | 0.85 | % | SNI-01-2981-1992 |
| Protein | 9.24 | % | SNI-01-2981-1992 |
| Tannin (phenolic acid) | 881.2 | mg/l | AOAC 952.03.2000 |

3.2.2. Carbohydrate and fat

Carbohydrate content in starter of yoghurt are 5.73%. The function of carbohydrate are source of energy and taste sweet on fruity. We feel fresh after drink yoghurt because we consumed of carbohydrate. Fat content in starter of yoghurt are 0.85%. Function fat similar carbohydrate as source of energy. Energy content on 100 gram fresh persimmons fruits 126 kcal [2]. Persimmons are delicious and exotic fruits that do more than serve as a sweet and tasty treat. Like most fruits, persimmons are a good source of fiber, containing almost 20% of the daily requirement in a single serving.

Fiber helps the body process food in a more efficient way, by adding bulk to the stool, stimulating peristaltic motion to move the food through the digestive tract, increase secretions of gastric and digestive juices, and relieve symptoms of constipation and diarrhea. Overall, a high-fiber fruit like persimmons can be a major boost to your gastrointestinal system, and can protect you from colorectal cancer and other similar diseases. It can also help people lose weight by defending against lipid uptake, which can cause obesity [1].

A diet fortified with dry persimmon peel is more efficient than the same diet fortified with dry persimmon pulp. Therefore the persimmon peel showing the effectiveness of its antioxidant activity can be used by individual consumers and in industrial processing [8].

The aim of this study was to compare the hypo cholesterolemic and antioxidant effects of two diets supplemented with dry persimmon in rats fed cholesterol (C). Three groups of male Wistar rats each of 13 animals during 4 weeks were fed different diets: the control group (CG)-semi purified diet with 1% of C and two experimental groups (EG1) and (EG2)-the same diet fortified with 7% of dry persimmon peel and dry persimmon pulp, respectively. In animals of all three groups before and after the 4-week trial period total cholesterol (TC), LDL-C, HDL-C,

triglycerides (TG), and lipid peroxides (LP) were examined. After the completion of the experiment a statistically significant increase in plasma TC and LDL-C in all three groups was found. In the animals of EG1 this increase was statistically less significant than in CG ($P < 0.05$ and $P < 0.025$ for TC and LDL-C, respectively). A statistically significant increase in the level of HDL-C was observed. The smallest one was in EG1. But only in the EG1 the HDL-C/TC ratio was increased more significantly (from 0.56 to 0.59). In EG1 a statistically less significant increase in LP than in CG ($P < 0.01$) was registered. The present results demonstrate that persimmon fruit exercises a hypo cholesterolemic and antioxidant effects and therefore is considered for an anti atherosclerotic diet.

3.2.3. Protein

Protein content is starter of yoghurt 9.24%. This is relative high. Protein very important for cell regeneration. They have a wealth of health benefits packed inside them, including their ability to improve eye health, reduce signs of aging, prevent various types of cancer, improve digestion, boost your immune system, lower cholesterol, increase your metabolism, strengthen your bones, boost cognitive function, lower blood pressure, and take care of your skin. Furthermore, they can help your body heal faster, aid in weight loss, reduce inflammation and increase blood circulation throughout the body [5].

3.2.4. Tannin (phenolic acid)

Tannin content in starter yoghurt 881.2 mg/l. The biosynthesis process of phenolic compounds in plants is summarized, which include the shikimate, pentose phosphate and phenyl propanoid pathways. Plant phenolic compounds can act as antioxidants, structural polymers (lignin), attractants (flavonoids and carotenoids), UV screens (flavonoids), signal compounds (salicylic acid, flavonoids) and defense response chemicals (tannins, phytoalexins). From a human physiological standpoint, phenolic compounds are vital in defense responses, such as anti-Aging, anti-inflammatory, antioxidant and anti-proliferative activities.

Therefore, it is beneficial to eat such plant foods that have a high antioxidant compound content, which will cut down the incidence of certain chronic diseases, for instance diabetes, cancers and cardiovascular diseases, through the management of oxidative stress. Furthermore, berries and other fruits with low-Amylase and high-glucosidase inhibitory activities could be thought of as candidate food items in the control of the early stages of hyperglycemia associated with type 2 diabetes [6].

The anti-oxidant activity of persimmon fruit appears to be mainly due to its high-molecular-weight tannin content. Antioxidant activity is variety specific with some astringent varieties showing very high antioxidant activity, comparable to strawberry and blueberry. In vitro, and limited animal studies, have shown that condensed tannins in the fruit may reduce the risk of cardiovascular disease, hypertension, diabetes and a wide range of cancers. Persimmon has an unusual property in that it appears to alter and reduce the rate of alcohol absorption and metabolism and thus ameliorate the symptoms of a hangover. The health and medicinal benefits of persimmon are considerable and should be further researched and promoted by persimmon industries around the world [7].

**3.3. Vitamin Analysis**

Vitamin C content 11.85 mg/100 gram. Persimmons have high levels of vitamin C, as well as phenolic compounds like catechins and gallocatechins, which are directly connected to preventing various types of cancer. Therefore, adding persimmons to your diet can help you stay protected from various types of cancer. This delicious little fruits is packed with anti-cancer agents that can boost your body's ability to fight free radicals. Free

Table 2. Analysis Vitamin Nutritional Value of Persimmons Liquid Auto Fermentation

| Parameter | The Result of Analysis | Unit of Measure | Methods |
|---|---|---|---|
| Vitamin C | 11.85 | mg/100 gram | Titrimetri |
| Vitamin B1 | 4864.23 | mg/kg | HPLC |
| Vitamin B2 | 85,87 | mg/kg | HPLC |
| Vitamin B6 | 0 | mg/kg | HPLC |

radicals are the byproducts of cellular metabolism that can mutate healthy cells into cancerous ones and damage various organ systems.

Antioxidants, which persimmons are packed with, seek out free radicals and eliminate them from the body, thereby improving overall health and protecting against a variety of diseases. In a related quality to the antioxidant and cancer prevention properties of persimmons, they also boost the immune system considerably.

Persimmons have one of the highest ascorbic acid (vitamin C) contents of any fruit, and a single persimmon has approximately 80% of the daily requirement of that beneficial nutrient. Vitamin C stimulates the immune system and increases the production of white blood cells, which are the primary line of defense for the body against microbial, viral, and fungal infections, as well as foreign bodies or toxins [1].

Along with the antioxidant properties that reduce the chances of cancer, you can also lower your health risks of developing tumors. Persimmons contain betulinic acid, which is a proven anti-tumor compound. This can reduce the chances of contracting tumors by inducing apoptosis, also known as programmed cell death, and if you already have a tumor, it can reduce the size and stop the cancer from metastasizing Anti –Aging Properties**:** Persimmons are rich in a number of vitamins, specifically vitamin A, beta-carotene, lutein, lycopene, and cryptoxanthins. These can all function as antioxidants in the body as well, specifically to reduce oxidative stress and prevent signs of premature aging, like wrinkles, age spots, Alzheimer's disease, fatigue, loss of vision, muscles weakness, and a number of other conditions.

Eye Health: Some of the compounds in persimmons also have a proven benefit for the health of your eyes! Zeaxanthin, a member of the B complex of vitamins, is directly linked to improved eye health due to its behavior as an antioxidant substance. Studies show that it reduces macular degeneration, cataracts, and night blindness.Metabolic Activity: Persimmons contain elements of the B complex of vitamins like pyridoxine, folic acid, and thiamin, which are all essential parts of various enzymatic processes and metabolic functions throughout the body, so keeping high levels maintained means that your body's systems will function efficiently and effectively, thereby increasing your overall metabolism. This can boost energy levels, increase muscle tone, improve digestion, and boost the immune system [1].Reactive oxygen species (ROS) and reactive nitrogen species (RNS, e.g. nitric oxide, NO{radical dot}) are well recognized for playing a dual role as both deleterious and beneficial species. ROS and RNS are normally generated by tightly regulated enzymes, such as NO synthase (NOS) and NAD(P)H oxidase isoforms, respectively.

Overproduction of ROS (arising either from mitochondrial electron-transport chain or excessive stimulation of NAD(P)H) results in oxidative stress, a deleterious process that can be an important mediator of damage to cell structures, including lipids and membranes, proteins, and DNA. In contrast, beneficial effects of ROS/RNS occur at low/moderate

concentrations and involve physiological roles in cellular responses to noxia, as for example in defence against infectious agents, in the function of a number of cellular signalling pathways, and the induction of a mitogenic response. Ironically, various ROS-mediated actions in fact protect cells against ROS-induced oxidative stress and re-establish or maintain "redox balance" termed also "redox homeostasis". The "two-faced" character of ROS is clearly substantiated. For example, a growing body of evidence shows that ROS within cells act as secondary messengers in intracellular signalling cascades which induce and maintain the oncogenic phenotype of cancer cells, however, ROS can also induce cellular senescence and apoptosis and can therefore function as anti-tumourigenic species. This review will describe the: (i) chemistry and biochemistry of ROS/RNS and sources of free radical generation; (ii) damage to DNA, to proteins, and to lipids by free radicals; (iii) role of antioxidants (e.g. glutathione) in the maintenance of cellular "redox homeostasis"; (iv) overview of ROS-induced signaling pathways; (v) role of ROS in redox regulation of normal physiological functions, as well as (vi) role of ROS in pathophysiological implications of altered redox regulation (human diseases and ageing). Attention is focussed on the ROS/RNS-linked pathogenesis of cancer, cardiovascular disease, atherosclerosis, hypertension, ischemia/reperfusion injury, diabetes mellitus, neurodegenerative diseases, rheumatoid arthritis, and ageing. Topics of current debate are also reviewed such as the question whether excessive formation of free radicals is a primary cause or a downstream consequence of tissue injury [9].

3.4. Mineral Analysis

Mineral very important to homeostatic process, regeneration red blood cell and white blood cell and blood circulation. Mineral content of starter 0.48 % consist of Cu 0.48 mg/l, Ca 154.25 mg/l, Fe 3.18 mg/l, Mg 149.87 mg/l, Zn 1.75 mg/l, Ni 0.24 mg/l, Pb 1.37 mg/l, Cr 0.25 mg/l.

Blood Circulation: Along with lower blood pressure, persimmons also provide Fe an essential element in creating new red blood cells. Without copper, you cannot uptake carious essential nutrients to make additional hemoglobin.

Increased circulation of healthy red blood cells increases cognitive function, muscle tone, metabolism, and energy levels, as well as wound repair and cellular growth. Persimmons also contain various vasodilating organic compounds that further drop blood pressure, making it a very good fruit for heart health [1].

3.5. Persimmon extract

Persimmon can decrease human lymphoid leukemia. Achiwa *et.all.* research have investigated the effects of persimmon (*Diospyros kaki*) extract (PS) and related polyphenol compounds such as catechin (C), epicatechin (EC), epicatechingallate (ECG), epigallocatechin (EGC), and epigallocatechingallate (EGCG) on the growth of human lymphoid leukemia Molt 4B cells. We found that PS, ECG, EGC, and EGCG strongly inhibited the growth of the cells in a dose-dependent manner, while C and EC inhibited the growth of the cells only moderately. Ornithine decarboxylase (ODC), a rate-limiting enzyme of polyamine biosynthesis, was inhibited by 10-20% by these polyphenol compounds. The morphology of the Molt 4B cells indicated severe damage 3 days after treatment with PS, ECG, EGC, and EGCG. Irregular shape of the cells and DNA fragmentation were observed in PS, ECG, EGC, or EGCG-treated cells. These results suggest that PS, ECG, EGC, and EGCG induce apoptosis (programmed cell death) of Molt 4B cells [10].

Table 3. Analysis Mineral Nutritional Value of Persimmon Liquid Auto Fermentation

| Parameter | The Result of Analysis | Unit of Measure | Methods |
|---|---|---|---|
| Mineral | 0.48 | % | SNI-01-2981-1992 |
| Al | 0 | mg/l | AAS |
| Cr | 0.25 | mg/l | AAS |
| Cu | 0.48 | mg/l | AAS |
| Ca | 154.25 | mg/l | AAS |
| Fe | 3.18 | mg/l | AAS |
| Mg | 149.87 | mg/l | AAS |
| Zn | 1.75 | mg/l | AAS |
| Ni | 0.24 | mg/l | AAS |
| Cd | 0 | mg/l | AAS |
| Pb | 1.37 | mg/l | AAS |

## Conclusion

Persimmons Yoghurt is a healthy soft drink and very good to maintenance our healthy and fitness as a naturally soft drink.

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## References

[1] 2016. Nutrition-and-you.com . [On line]. Available http://www.nutrition-and-you.com/index.html

[2] 2016. Wikipedia. [On line]. Available https://en.wikipedia.org/wiki/Condensed_milk

[3] 2016. Health Hype.com. [On line]. Available http://www.healthhype.com/salmonella-and-shigella-differences-causes-disease-symptoms.html

[4] 2016. Centre of Disease Control and Prevention. [On line]. Available https://www.cdc.gov/ecoli/

[5] 2016. Organic Fact. [On line]. Available https://www.organicfacts.net/health-benefits/fruit/persimmons.html

[6] D. Lin, D. M. Xiao, M. L. Zhao, J. Z. Li, Z. B. Xing, B. X. Li, X. M. Kong, M. I. Li, Q. Zhang, Y. Liu, Y, H. Chen, W. Qin, H. Wu, S. Chen, An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes, *Molecules*. Vol 21. Issue 10. Article Number 1374. October 2016.

[7] A. P. George, S. Redpath, Health and medicinal benefits of persimmon fruit: A review, *Advances in Horticultural Science*. Vol 22. Issue 4. pp 244-249. 2008.

[8] S. Gorinstein, G. W. Kulasek, F. Bartnikowska, M. Leontowicz, M. Zemser, M. Morawiec, S. Trakhtenberg, The influence of persimmon peel and persimmon pulp on the lipid metabolism and antioxidant activity of rats fed cholesterol. *Journal of Nutritional Biochemistry*. Vol 29. Issue 4. Pp 223-227. April 1998.

[9] M. Valko, D. Leibfritz, I. Moncol, M. T. D. Cronin, M. Mazur, I. Telser, Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry and Cell Biology*. Vol 39. Issue 1. pp 44-84. 2007.

[10] Y. Taguchiwa, H. Hibasami, H. Katsuzaki, K. Imai, T. Komiya, Inhibitory Effects of Persimmon (*Diospyros kaki*) Extract and Related Polyphenol Compounds on Growth of Human Lymphoid Leukemia Cells, *Bioscience, Biotechnology and Biochesmistry*. Vol 61. Issue 7. Pp 1099-1101.

[11] C.G. Moertel, T.R. Fleming, E.T. Creagan, J. Rubin, M.I. O'Connell, M. M. Ames, "High-dose vitaminC versus placebo in the treatment of patients with advanced cancer who have had no prior chemotherapy. A randomized double-blind comparison", *New England Journal of Medicine*. Vol 312. Issue 3. pp 137-141. 1985.

[12] F. Pu, X. L. Ren, X. P. Zhang, Phenolic compounds and antioxidant activity in fruits of six *Diospyros kaki* genotypes, *Europian Food Research and Technology*. Vol 237. Issue 6. pp 923-932. December 2013.

[13] G. Iolascon, R. Gimigliano, M. Bianco, M., A. de Sire, A. Moretti, A. Giusti, N. Malavolta, S. Migliaccio, A. Migliore, N. Napoli, P. Piscitelli, G. Resmini, U. Tarantino, F. Gimigliano, Are dietary supplements and nutraceuticals effective for musculoskeletal health and cognitive function? A scoping review, *Journal of Nutrition, Health and Aging.* Page 1-12. October 2016.

[14] M. Farooqui, R. Pardeshi, S. Jadhav, Antioxidant-vitamin C: Lung function; lung cancer. *Asian Journal of Pharmaceutica and Clinical Research*. Vol 9. pp 43-51. October 2016.

[15] M. Rejeb, R. Sadraoui, T. Najar, Role of vitaminC on immune function under heat stress condition in dairy cows. *Asian Journal of Animal and Veterinary Advances.* Vol 11. Issue 11. pp 717-724. 2016.

[16] N. Mikirova, I. Casciari, A. Rogers, P. Taylor, "Effect of high-dose intravenous vitaminC on

inflammation in cancer patients", J*ournal of Translational Medicine*, Vol 10. Issue 1. Artikel Number 187. 11 September 2012.

[17] N. Mikirova, R Hunnunghake, R.C. Scimeca, C. Chinshaw, F. Ali, C. Brannon, N. Riordan, "High-dose intravenous vitamin C treatment of a child with neurofibromatosis type 1 and optic pathway glioma: A case report ", *American Journal of Case Report*, Vol 17, pp 774-781, 24 October 2016.

[18] S. Snekalatha, Effect of Ascorbic acid on Interleukin-2 secretion by T Lymphocytes in Whole Blood Cultures, *Indian Journal of Physiology and Pharmacology*. Vol 60. Issue 2 . pp 205-207. 2016.

[19] 2016. Fittness World. [On line] Available http://wiki-fitness.com/persimmon-fruit-health-benefits-and-nutrition-facts/

# Evaluated the effect of fermented palm sludge on burgo chicken performance

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## Abstract

Bengkulu has a potential source of feed material as well as its availability is met throughout the year, and has been tested to animals is the oil sludge. Oil sludge can be obtained with the cheap price and the protein content is high enough. This research was aimed to evaluate the effect of fermented palm sludge (FPS) with *Neurospora sp* on burgo chicken performance. It was doing on 21 Desember 2014 until 15 Februari 2014 in Pagar Dewa, Kecamatan Selebar, Kota Bengkulu. This used 16 burgo chickens age 8-9 month that groups in 4 treatments with 4 samples that placed in individual cage. Treatments were P0 (diet control), P1 (diet with 5% FPS), P2 (diet with 10% FPS) dan P3 (diet with 15% FPS). Result showed that FPS did not improve ration consumption, did not reduce egg production percentation (%), did not reduce egg production (grain), did not reduce egg massa production (gram) and did not improve ration convertion ($P>0.05$). The conclusion is fermented palm sludge until 15% in diet do not improve ration consumption, did not reduce egg production percentation (%), do not reduce egg production (grain), do not reduce egg massa production (gram) and do not improve ration convertionof burgo chicken. So, fermented palm sludge can use as diet until 15% and do not reduce performance of burgo chicken

Keywords: Fermented palm sludge, Burgo chicken

## 1. Introduction

Eggs are farm products that contribute to the achievement of adequate nutrition in the community. An egg obtained adequate nutrition perfect because eggs contain nutrients that are very good and easily digested by the body (Sudaryani, 2003). Lately symptoms of back to nature into something interesting. Upper middle class people who originally liked everything based technology is now starting to change to the situation that all natural. Demand for eggs and chicken eggs increased apparently participated affected by the phenomenon. Public perception of the chicken is the original chicken reared traditionally and are not given feed containing chemicals. Potential local chicken, which can be developed in the area of Bengkulu is burgo chicken that has huge potential and has a characteristic that is different from the other chickens (Putranto, 2011). Hen burgo can lay 20-25 point range in the period (Setianto, 2009). Lapse of time laying the average of 10 days, it is much faster than domestic poultry in general, which has an interval of spawning an average of 18 days.

In other side, Bengkulu has a potential source of feed material as well as its availability is met throughout the year, and has been tested to animals is the oil sludge. Oil sludge is a waste generated from extortion palm fruit that has been through various processes to produce crude palm oil (CPO) or commonly known as crude palm oil (Sinurat, 2003). Oil sludge can be obtained with the

cheap price and the protein content is high enough. Protein content in the sludge of oil that has been fermented using a mold Neurosprora sp increased from 13.57% to 23.45 (Fenita et al, 2010). The granting of oil sludge fermentation (LSF) of 15% does not give negative effects to birds, especially for the performance of domestic poultry (Sinurat, 2001). Expected use of oil sludge that has been fermented with Neurosprora sp could be expected to improve the performance of chicken burgo.

This study aimed to evaluate the effect of oil sludge that has been fermented with Neurosprora sp against burgo chicken performance.

## 2. Material and Methods

It was doing on 21 Desember 2014 until 15 Februari 2014 in Pagar Dewa, Kecamatan Selebar, Kota Bengkulu. This used 16 burgo chickens age 8-9 month that groups in 4 treatments with 4 samples that placed in individual cage. Treatments were P0 (diet control), P1 (diet with 5% FPS), P2 (diet with 10% FPS) dan P3 (diet with15% FPS). And used analysis of Completely Randomized Design (RCD). When a result is significant, it continue to Ducan's Mutliple Range Test (DMRT) to analysis means test.

## 3. Result and Discussion

3.1 Feed Consumption

Average consumption of hen burgo rations grams/head/day during the study are shown in Table 1. Based on the research results feed intake during the study had no significant (P> 0.05). The use of oil sludge fermentation at the level of 15% in hen burgonot increase feed intake. It is the same with research of Sari et al. (2012), the use of LSF as much as 15% in laying chicken does not increase feed intake compared with control treatment.

Chicken consume rations is none other than to meet the basic needs of life, growth and reproduction (Tilman et al., 1989). Feed intake is strongly influenced by the environment and the balance of nutrients, the quality of rations, breeds, rate of growth, body weight and level of production. In selecting a feed, the chicken will use his instincts to choose the feed that is, if the feed contains enough nutrients then the level of palatability of the chicken was high on the contrary, if the feed is less containing nutrients required for the necessities of life, the chickens will continue to consume feed that meets every aspect of their daily needs. Increasing and decreasing feed intake is influenced by energy content, of this study on the energy content of the ration so that the same relative feed consumption showed no significant.

*3.2 Percentage of Egg Production*

The influence of the use of FPS against the percentage of egg production during the study showed no significant (P> 0.05), this can be seen in Table 1. The use of oil sludge fermentation to the extent of 15% did not increase egg production, but also does not reduce the percentage of egg production in burgo chicken. The use of sludge oil to the extent of 15% in laying chicken showed no negative symptoms (Sinurat, 2003). The use of FPS is not optimal to increase egg production due to FPS deficiency in amino acids lysine and mentionin (Fenita et al., 2010).

Although the treatment effect was not significant (P> 0.05) on the percentage of the average production during the study, but it can be seen that the percentage of egg production related to feed intake (Table. 1). In each treatment P0: 49.56 g/head/week (42.86), P1: 43.52 g/head/week (23.21), P3: 42.28g/head/week (28,57). The number of low feed intake can cause a drop in egg production and feed intake otherwise high can increase the amount of egg production [9] Amrullah, 2003.

Table 1. The result in this research

| Variables | Treatments | | | |
|---|---|---|---|---|
| | P0 | P1 | P2 | P3 |
| Feed Consumption (g/head/day) | 53.46±4.34 | 53.62±9.19 | 55.46±6.32 | 60.91±12.82 |
| Percentage of Egg Production (%) | 51.34±9.82 | 45.09±16.00 | 41.07±10.00 | 51.34±16.58 |
| Egg Production (grain/head) | 28.75±5.50 | 25.25±8.96 | 23.00±5.60 | 28.75±9.29 |
| Production of Egg Mass (g/head) | 765.96±134.49 | 734.08±288.78 | 648.01±181.72 | 810.90±264.79 |
| Feed Convertion | 3.98±0.61 | 4.47±1.37 | 4.96±0.84 | 4.41±0.92 |

*3.3 Egg Production (Grain)*

Results of analysis of variance showed that the use of FPS against egg production (grain) during penilitian not significant (P> 0.05). According to Prasetyo and Ketaren (2005) ration quality and environmental conditions greatly affect the number of eggs produced. Rations with low protein content are not capable of supporting high egg production. The protein content of the ration in this study are relatively the same, so do not reduce the production of eggs. In this research use of oil sludge fermentation at 5%, 10%, and 15% do not reduce the production of eggs.

Maintenance aspects affect performance both in terms of consumption, production and reproduction. The chickens are kept intensively necessities of life governed by the breeder, unlike chickens are reared extensively dependent to ambient conditions both feed and place of residence. In this study burgo chickens can produce 23-28 eggs per period, it agrees with Warnoto (2002) which suggests that the maintenance of burgo intensive chicken can increase the amount of egg production per period.

*3.4 Production of Egg Mass*

Results of analysis of variance totaling mass production of chicken eggs burgo does not affect the provision of FPS to the extent of 15% during the study (Table 1). An important factor influencing the size of the egg is protein and amino acids, about 50% of dry matter so that eggs contain protein provides amino acids in protein synthesis is very necessary for the production (Anggorodi, 1994). According to Montesqrit (2007) linoleic acid content contained in the ration able to sustain the weight of eggs produced by laying hens. The balance of amino acids and linoleic acid contained in the study, is quite balanced rations that do not cause a decrease in egg mass production.

*3.5 Feed Convertion*

Feed conversion was calculated to add the feed consumed divided by the total weight of eggs produced. Based on the study results during burgo chicken feed conversion in treatment P0, P1, P2 and P3 during the study showed no significant (P> 0.05). Rasyaf (2003) explains that the feed conversion rate is the number of ration consumed a chicken in a certain time to form a meat or eggs. Factors affecting feed conversion rate among other strain, feed quality, state of the cage and gender. Based on this study feed quality is relatively the same, so do not improve feed conversion and also does not reduce the value of feed conversion (Anggorodi, 1994). Use of FPS to the extent of 15% does not improve the feed conversion but the use of LSF to some 15% also does not reduce the value of feed conversion, so the use of LSF in burgo chicken feed could be used to the extent of 15%.

**Conclusion**

It can be concluded that the use of oil sludge fermentation in the ration to the extent of 15% does not decrease feed consumption, egg production and feed conversion burgo chicken. Utilization of oil sludge fermentation can be used as chicken feed burgo to the

extent of 15% without lowering the production performance of burgo chicken.

## References


[1] Sudaryani T. 2003. Kualitas telur. Cetakan Keempat. PT Penebar Swadaya (ID): Jakarta

[2] Putranto, H. D. 2011. Pengaruh Suplementasi daun katuk terhadap ukuran ovarium dan oviduk serta tampilan produksi telur ayam Burgo. Jurnal Sain Peternakan Indonesia, 2, 103-114.

[3] Setianto J. 2009. Ayam Burgo Ayam Buras Bengkulu. IPB Press. Bogor.

[4] Sinurat A. P. 2003. pemanfaat lumpur sawit untuk bahan pakan unggas. Wartazoa, 13, 39-47.

[5] Fenita, Y., Santoso, U, and Prakoso, H. 2010. Pengaruh lumpur sawit fermentasi dengan *Neurospora sp* terhadap performans produksi dan kualitas telur. Jurnal Ilmu Ternak dan Veteriner, 2, 88-96.

[6] Sinurat, A. P., Purwadaria, Pasaribu, T., Darma, J., Bintang, I. A. K., and Torgatop, M. H. 2001. Pemanfaatan lumpur sawit fermentasi untuk ransum unggas. Penggunaan produk lumpur sebelum dan sesudah dikeringkan dalam ransum ayam kampung yang sedang tumbuh. Jurnal Ilmu Ternak Veteriner, ….., ….. - …..

[7] Sari, R. P, Kaharuddin, D., Santoso, U., and Fenita, Y. 2012. Pengaruh suplementasi ekstrak daun katuk (*Sauropusandrogynus*) dalam ransum berbasis lumpur sawit fermentasi terhadap performans ayam ras petelur. Jurnal Sains Peternakan Indonesia, 2, 81-92.

[9] Amrullah, I. K. 2003 Nutrisi ayam petelur. Lembaga Gunung Budi (ID): Bogor.

[10] Prasetyo, H., and Ketaren, P. 2005. Interaksi antar bangsa itik dan kualitas ransum pada produksi dan kualitas telur itik lokal. Seminar Nasional Teknologi Peternakan dan Veteriner, 3, 230-235. Balai Penelitian Ternak (ID): Bogor.

[11] Warnoto. 2002. Transformasi genetik ayam burgo dalam rangka penyediaan bibit dan peningkatan produksi telur ayam kampung di Bengkulu. Jurnal Pengembangan dan Penerapan Teknologi, …, …. - ….

[12] Anggorodi. 1994. Ilmu makanan ternak umum. Gramedia (ID): Jakarta.

[13] Montesqrit. 2007. Penggunaan bahan pakan sebagai penyalut dalam mikroenkapsulasi minyak ikan lemuru dan pemanfaatannya dalam ransum ayam petelur [Disertasi]. Sekolah Pasca Sarjana. Institut Pertanian Bogor: Bogor.

[14] Rasyaf M. 1994. Makanan ayam Broiler. Kanisius (ID): Yogyakarta.

# In-Vitro Rumen Digestibility of Goat Feed by Patikan Kerbau (*Euphorbia hirta* L.) Herbal Supplemented

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## Abstract

The purpose of this research was to find the digestibility of dry matter, organic matter, concentration of VFA and N-$NH_3$ on goat feed by patikan kerbau (*Euphorbia hirta* L.) herbal supplemented. The material of this research was goat rumen fluid, feed goat and patikan kerbau herbal. This research was designed using 6 treatments and 4 groups as replication by randomized block design (RBD). The treatment of this research was herbal supplementation level in the ration with the following ration formulation. P0 = 60% forage and 40% concentrate, P1 = P0 + 5% patikan kerbau, P2 = P0 + 10% patikan kerbau, P3 = P0 + 15% patikan kerbau, P4 = P0 + 20% patikan kerbau and P5 = P0 + 25% patikan kerbau. Parameter observed in this study includes the digestibility of dry matter, organic matter, concentrations of total VFA and N-$NH_3$. The Anova showed that supplementation of patikan kerbau provided non significant influence ($P>0.05$) on ruminal pH, dry matter digestibility, concentrations of total VFA and N-$NH_3$, but significant influence ($P<0.05$) on organic matter digestibility. The conclusion from this research that supplementation of herbal patikan kerbau (*Euphorbia hirta* L.) in the goat ration can increase dry matter digestibility, organic matter digestibility, the concentrations of total VFA and N-$NH_3$ up to level 15%.

Keywords: in-vitro digestibility, patikan kerbau (Euphorbia hirta L.), supplementation, goat rumen fluid

## 1. Introduction

The ability of milk production of PE goat is still very diverse, ie 0.45 to 2.1 liter/day. Many ways in which to increase milk production, in addition to feeding the right, supplementation with feed additives in the form of synthetic compounds also carried, whose main goal to increase the flow of substrate to the mammary gland and also increase the number of secretory cells of the mammary gland. But the indiscriminate and prolonged use of that synthetic compounds can cause side effects that can reduce productivity, immune and reproductive performance in animals. At present, development of herbal galactogogues is important in safe milk production. The use of galactogogues of herbal origin seems to reaffirm the milch-herd health without causing deleterious alterations in the tissue reactions (Mishra *et al*., 2006), and have no side effects and do not leave residues in tissues, secretions, excretions and milk (Behera *et al*., 2013). One of the medicinal plants that have galactogogue properties are herbal patikan kerbau.

Patikan kerbau (E. hirta) is a wild plant that is found in the tropics, has a reputation to increase milk flow in women and when administered to female rabbits before puberty can enhance the development of the mammary gland and causes secretion. The ability of herbal patikan kerbau to improve milk production involving the active

compounds Euphorbine A dan Euphorbine B, which have the property of increasing the secretion of prolactin (the principal hormone of lactation) and of increasing the secretion of β-casein (the principal protein of milk) and, as a consequence, they increase the secretion of milk in women and animals (Nguyen, 1990).

Results of research by Mihardja et al. (2001) which tested the lactogogue activity of the extract patikan kerbau in mother rats with a dose of 812.4 mg, 81.24 mg and 8.124 mg / 100 g body weight showed that the extract of patikan kerbau significantly increased the pups weight at 81.24 mg treatment group, but did not significantly increase the volume of milk and prolactin mother rats compared with controls. No significant increased in the volume of milk was apparently due the difficulty of measuring the level of milk production in mice, and usually milk yield estimations for rats by means of pup weight and weight gains (Lompo-Ouedraogo et al., 2004). Administration at a dose of 812.4 mg/100 weight and 8.124 mg/100 g body weight did not show effectiveness, the possibility of high doses, can damage the liver, so that the metabolism of nutrients is interrupted, whereas small doses did not provide sufficient effect (Mihardja et al., 2001).

The use of plants patikan kerbau as a feed supplement to increasing milk production in ruminants has not been done. In the field of nutrition of ruminants, testing the effects of feeding is often done through in-vitro experiments. This experiment is intended to obtain preliminary information on the effect of feed on rumen bioprocess. Additionally, this test can also predict response livestock productivity, when you get a ration with a particular feed composition.

Based on the explanation above, this study aims to assess supplementation of herbal patikan kerbau in rations toward the in-vitro rumen ecology (pH, dry matter digestibility, organic matter digestibility, the concentration of total VFA and N-$NH_3$ rumen fluid).

## 2. Material and Methods

The materials of this study were the rumen fluid goats, feed the goats consisting of 60% forage and 40% concentrates, which is the ration of basal, herbal patikan kerbau (*Euphorbia hirta* L.), a solution McDougall's buffer, the device analyzes the concentrations of VFA, N-$NH_3$, dry matter and organic matter and pH.

This study was designed using a randomized block design (RBD) with 6 treatments and 4 groups goat rumen fluid as replication. The treatment of this research was herbal supplementation level of herbal patikan kerbau (*Euphorbia hirta* L.) in the ration with the following ration formulation. P0 = 60% forage and 40% concentrate, P1 = P0 + 5% patikan kerbau, P2 = P0 + 10% patikan kerbau, P3 = P0 + 15% patikan kerbau, P4 = P0 + 20% patikan kerbau and P5 = P0 + 25% patikan kerbau.

The parameters measured were pH, VFA concentration, N-$NH_3$ concentration, digestibility of dry matter and organic matter of goats rumen fluid.

The data obtained in this study were analyzed by analysis of variance, if there were significantly different results ($P<0.05$) among the treatments, then continued with Duncan test at 5% level (Steel and Torrie, 1991).

## 3. Result and Discussion

Rumen fluid characteristics of goat supplemented with patikan kerbau can be seen in Table 1.

### 3.1. Ruminal pH

Results of analysis of variance showed that supplementation patikan kerbau into rations had no significant effect ($P> 0.05$) on pH rumen fluid goat. This is thought by administration patikan kerbau did not interfere with the process of digestion of feed by microbial activity in the rumen.

Table 1. Characteristics of Goat Rumen Fluid Supplemented with Patikan Kerbau

| Characteristics of Rumen Fluid | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | P0 | P1 | P2 | P3 | P4 | P5 |
| pH | 6,82 | 6,81 | 6,81 | 6,86 | 6,95 | 6,87 |
| KCBK (%) | 58,67 | 56,89 | 58,09 | 60,14 | 58,97 | 53,55 |
| KCBO (%) | 60,62[b] | 59,81[b] | 61,63[b] | 63,37[b] | 62,66[b] | 52,83[a] |
| VFA (mM) | 57,36 | 56,06 | 64,79 | 69,65 | 69,50 | 69,43 |
| N-NH3 (mM) | 9,14 | 9,03 | 11,44 | 11,97 | 6,03 | 4,81 |

Description: Different superscript on the same row indicate significantly different (P <0.05).

The pH values obtained from this study (Table 1) was still within the normal ranges for rumen microbial activity. Johnson (1966) stated that the optimum pH to maintain the vitality of microbes in the rumen ranges from 6.7 to 7.0, to digest cellulose 6.9 and to starch 6.8.

3.2. Dry Matter Digestibility

In-vitro digestibility determined by calculating the post-process residues incubation for 48 hours. High dry matter digestibility in ruminants showed high digestible nutrients mainly digested by rumen microbes. The higher the percentage digestibility of the feed material, means the better the quality. The value of the ration dry matter digestibility of each treatment in this study are listed in Table 1.

Results of analysis of variance showed that supplementation of patikan kerbau into rations had no significant effect (P> 0.05) on the dry matter. Ration dry matter digestibility of this study was still in the normal range as stated by Schneider and Flatt (1975), that the normal range is 50.7 to 59.7% dry matter.

3.3. Organic Matter Digestibility

Digestibility of organic matter is closely related to dry matter, because most of the dry material composed of organic material (Sutardi, 1981). A decrease in dry matter digestibility would result in reduced digestibility of organic matter or vice versa (Syaro et al., 2005). Results of analysis of variance showed that supplementation of patikan kerbau into rations had significantly effect (P <0.05) on organic matter digestibility. Duncan test showed that supplementation of 25% patikan kerbau (P5) lowering the organic matter digestibility was 52.83%, whereas between treatments supplementation of 0% (P0), 5% (P1), 10% (P2), 15% (P3) and 20% (P4) did not differ significantly. Supplementation patikan kerbau as much as 15% (P3) provides organic matter digestibility highest of 63.37%.

3.4. Levels of VFA

Volatile fatty acids (VFA) is the end product of fermentation of carbohydrates and main energy source for ruminants (Parakkasi, 1999). Total VFA levels of each treatment in this study are listed in Table 1. Based on the analysis of variance, it appears that treatment of supplementation of patikan kerbau into rations had no significant effect (P> 0.05) on the VFA levels.

The mean levels of total VFA results of this study ranged from 56.06 to 69.65 mM, and tends to increase along with the increasing standard of the patikan kerbau supplementation up to 15% and then decreased with increasing the level up to 25%. Production of VFA resulting in the rumen is highly variable, namely in the range of 70-150 mmol/liter, depending on the type of diet consumed (McDonald et al., 2010). Sutardi (1977) stated that the VFA levels needed to support optimal rumen microbial growth is 80-160 mM.

3.5. Levels of N-NH3

Based on the analysis of variance showed that the ration treatment effect was not significant (P> 0.05) on levels of N-NH3. N-NH3 levels reflect the amount of dietary protein in the rumen and the value is highly influenced by the ability of rumen microbes to degrade protein ration (Prihandono, 2001 in Muhtarudin and Liman, 2006).

The mean levels of N-NH3 results of this study ranged from 4.81 to 11.97 mM and within normal limits, but tended to increase with increasing level of supplementation patikan kerbau up to 15% and then decreased with increasing the level up to 25%. Satter and Slyter (1974) states that the growth of rumen microbes began to fail when the levels of N-NH3 in the rumen around 3.57 mM. N-NH3 levels of rumen fluid that supports the growth of rumen microorganisms is 4-12 mM and N-NH3 levels optimum is 8 mM (Sutardi, 1977).

**Conclusion**

The results of this study concluded that the supplementation of herbal patikan kerbau (Euphorbia hirta L.) in the ration of goats in vitro rumen bioprocess not interfere until the level of 15% and can increase the digestibility of dry matter, organic matter, concentrations of total VFA and N-NH3.

**References**

Behera, P. C., Tripathy, D. P., and Parija, S. C. 2013. Shatavari : Potentials for galactogogue in dairy cows. Indian Journal of Traditional Knowledge, 12, 9-17.

Johnson, R. R. 1966. Techniques and Procedures for In Vitro and In Vivo Rumen Studies. J Anim Sci., 25, 855-875. http://www.journalofanimalscience.org/content/25/3/855.

Lompo-Ouedraogo, Z., D. van der Heide, E. M. van der Beek, H. J. M. Swarts, J. A. M. Mattheij and L. Sawadogo. 2004. Effect of aqueous extract of *Acacia nilotica* ssp *adansonii* on milk production and prolactin release in the rat. Journal of Endocrinology, 182, 257-266.

McDonald, P., Edwards, R. A., Greenhalgh, J. F. D., Morgan, C. A., Sinclair, L. A., and Wilkinson, R. G. 2010. Animal Nutrition. Seventh Edition. Prentice Hall, London (GB).

Mihardja, L., Adimunca, C., Widowati, L., Raflizar, Pujiastuti, Winarno and Wahjoedi, B. 2001. Manfaat ekstrak etanol patikan kebo (Euphorbia hirta L.) sebagai laktagogum pada tikus putih yang menyusui. But. Penelit. Kesehat., 29, 118-125.

Mishra, U. K., Kanesh, J. S., Mandal, A. K., Das, R. K., Rayaguru, K., and Parija, S. C. 2006. Potentials of herbal galaktogogum in milk production in ruminants. The Indian Cow July-September, 2006. Department of Anatomy, Histology & Embryology, Faculty of Veterinary Sciences & AH, Orissa University of Agriculture & Technology, Bhubaneswar–751 003 (Orissa).

Muhtarudin and Liman. 2006. Penentuan tingkat penggunaan mineral organik untuk memperbaiki bioproses rumen pada kambing secara *in-vitro*. Jurnal Ilmu-Ilmu Pertanian Indonesia, 8, 132-140.

Nguyen, T. H. 1990. Plant polysaccharide fractions inducing prolactin in mammals. United State Patent and Trademark Office No. US4948785.

Ørskov, E. R. and Ryle, M. 1990. Energy Nutrition in Ruminants. Elsevier Science Publisher Ltd., London.

Parakkasi, A. 1999. Ilmu Nutrisi dan Makanan Ternak Ruminansia. Universitas Indonesia Press, Jakarta.

Satter, L. D. and Slyter, L. L. 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. British Journal of Nutrition, 32, 199-208.

Schneider, B. H. and Flatt, W. P. 1975. The evaluation of feeds through digestibility experiments. University of Georgia Press.

Steel, R. G. D. and Torrie, J. H. 1991. Prinsip dan prosedur statistika. PT. Gramedia. Pustaka Utama. Jakarta.

Sutardi, T. 1977. Ikhtisar ruminologi. Bahan kursus peternakan sapi perah. Kayu Ambon. Dirjen Peternakan FAO. Halaman 55-60.

Sutardi, T. 1981. Sapi Perah dan pemberian makanannya. Departemen Ilmu dan Makanan Ternak. Fakultas Peternakan IPB. Bogor.

# The Adding of *Saccharomyces cerevisiae* on Moisture, Acidity and Lactic Acid Bacteria Colony Count of Yogurt from Goat's Milk

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## Abstract

The study of the *Saccharomyces cerevisiae* adding on goat's milk yogurt has been done in order to determine the effect of the *Saccharomyces cerevisiae* in tape yeast on moisture, acidity and lactic acid bacteria colonies count. The adding of *Saccharomyces cerevisiae* (yeast) in fermented milk can promote the growth and activity of lactic acid bacteria. In its activities the yogurt bacteria, namely *Lactobacillus bulgaricus* and *Streptococcus thermophilus* which is a homofermentatif bacteria, together with yeast (*Saccharomyces cerevisiae*) causes acid production faster and higher resulting protein coagulated followed by an increase in total solids of yogurt. This research method is an experiment with using a Block Randomized Design consisted of 6 treatments and 3 replications. The treatment is the adding of *Saccharomyces cerevisiae* (tape yeast) as much as: A (0%), B (0.25%), C (0.50%), D (0.75%), E (1%) and F (1.25%) into yogurt from goat's milk. The variable was observed the content of moisture, acidity and lactic acid bacteria colony count. The result of this research indicated that the adding of Saccharomyces *cerevisiae* (tape yeast) were increased the acidity and lactic acid bacteria colony count significantly ($P < 0.01$) and decreased the moisture content of goat's milk yogurt. The adding of yeast tape up to 1.25% is the best in producing goat's milk yogurt.

Keywords: *Saccharomyces cerevisiae*, yeast, yogurt, goat's milk

## 1. Introduction

Goat's milk is a white liquid produced by ruminants from the type of goat (Capra). Goat's milk has nutritional value is not inferior to the cow's milk nutrient content. It is contains calcium, phosphorus, and vitamins and can be used by people who are allergic to cow's milk or experiencing indigestion. Sodiq and Abidin (2008) stated that the chemical composition of goat's milk is generally no different from cow's milk. Goat's milk fat granule size between 1- 10 mm. The small size of fat granule cause goat's milk is more easily digested by the human digestion system, and does not cause diarrhea for those who consume them.

Culture of goat's milk drinking in Indonesia has not been so popular in the community, because goat's milk has a distinctive aroma that is less desirable. However, the smell of goat's milk can be eliminated by processing goat's milk into products that have added value without reducing the efficacy. One of the products processed from goat's milk is yogurt. Yogurt is a fermented dairy product containing two lactic acid bacteria, namely Streptococcus thermophilus and Lactobacillus bulgaricus (Helferich and Westoff, 1980). Both of these bacteria will hydrolysis milk sugar (lactose) into lactic acid so that the acidity of the milk is increase followed by a decrease in pH resulting milk protein coagulated to form a "curd" (Tammime and Robinson, 1989). Based on the Indonesian National Standard (SNI) for yogurt issued by the National

Standardization Agency (2009) is a yogurt with good quality contain lactic acid about 0.5 - 2.0%.

The addition of yeast or yeast in fermented milk can be done to improve the growth of lactic acid bacteria during fermentation. Yeast most commonly used is *Saccharomyces cerevisiae*. According to Surono (2004) fermentation products involving other lactic acid bacteria, but yeasts also responsible for the formation of alcohol and is known in Asia like koumiss, kefir. Hotnida (2003) states that the *Saccharomyces cerevisiae* often used in the manufacture of tape, so the tape yeast can be used as source of *Saccharomyces cerevisiae* which is cheap. The adding yeast in fermented milk can promote the growth and activity of lactic acid bacteria. Hidayat et al. (2006) stated that the concentration of the use of commercial yeast *(Saccharomyces cerevisiae)* that is commonly used is 0.2% and with a pure culture is 1%. In its activities *Lactobacillus bulgaricus* and *Streptococcus thermophilus* which is a bacteria homofermentatif, together with yeast (*Saccharomyces cerevisiae*) causes acid production faster and higher resulting milk protein coagulated, so that formation of yogurt is faster (Surono, 2004).

## 2. Material and Methods

The method in this study is an experimental method using a Block Randomized Design (RAK) consisted of 6 treatments and three replications. The treatment is the adding of *Saccharomyces cerevisiae* in yeast tape as much as A (0%), B (0.25%), C (0.50%), D (0.75%), E (1%) and F (1:25%) into the yogurt of goat's milk which has been given a *Lactobacillus bulgaricus* and *Streptococcus thermophillus* as much as 2%. The variable was measured the content of moisture, acidity and lactic acid bacteria colonies count of goat's milk yogurt. The procedure of this study is as follows:

One thousand and two hundred milliliter (1200 ml) of goat's milk is pasteurized at 65° C for 30 minutes in a water bath while stirring, then add 4% sugar. Then the temperature is lowered to 43oC and then inoculated *Lactobacillus bulgaricus* and *Streptococcus thermophillus* (1:1) as much as 2%, stirring frequently to be homogeneous. After that the milk is divided into six bottles each of 200 ml, which is then randomly divided into three treatment groups to add yeast tape containing *Saccharomyces cerevisiae* respectively of: A (0%), B (0.25%), C (0.50%), D (0.75%), E (1%) and F (1:25%). After homogeneous bottle was sealed and incubated in an incubator for 5 hours at 43°C. Yogurt produced is stored in a refrigerator at 5° C for observation corresponding measured variables.

When the treatments showed significantly different results ($P < 0.05$) then it will continue with the Duncans Multiple Range Test (DMRT), while the taste value were analyzed using non-parametric Friedman Test (Steel and Torrie, 2005).

## 3. Result and Discussion

### *3.1. Moisture*

Statistical analysis showed that the addition of *Saccharomyces cerevisiae* in tape yeast has reduced the moisture content of yogurt goat's milk significantly ($P < 0.01$), with a moisture content of yogurt lowest in treatment F (79.87%) followed by treatment of E (80.05%), D (80.53% ), C (81.00%), B (82.32%) and the highest in the treatment A (83.21%), as shown in Table 1. The reduced moisture content in goat's milk yogurt following the increasing of tape yeast, caused of the yeast tape containing *Saccharomyces cerevisiae*, which a role in increasing the activity of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in modifying lactose into lactic acid (Surono, 2004). In its activities the yogurt bacteria like *Lactobacillus bulgaricus* and *Streptococcus thermophilus* which is a homofermentatif bacteria, together with *Saccharomyces cerevisiae* causes acid production faster and

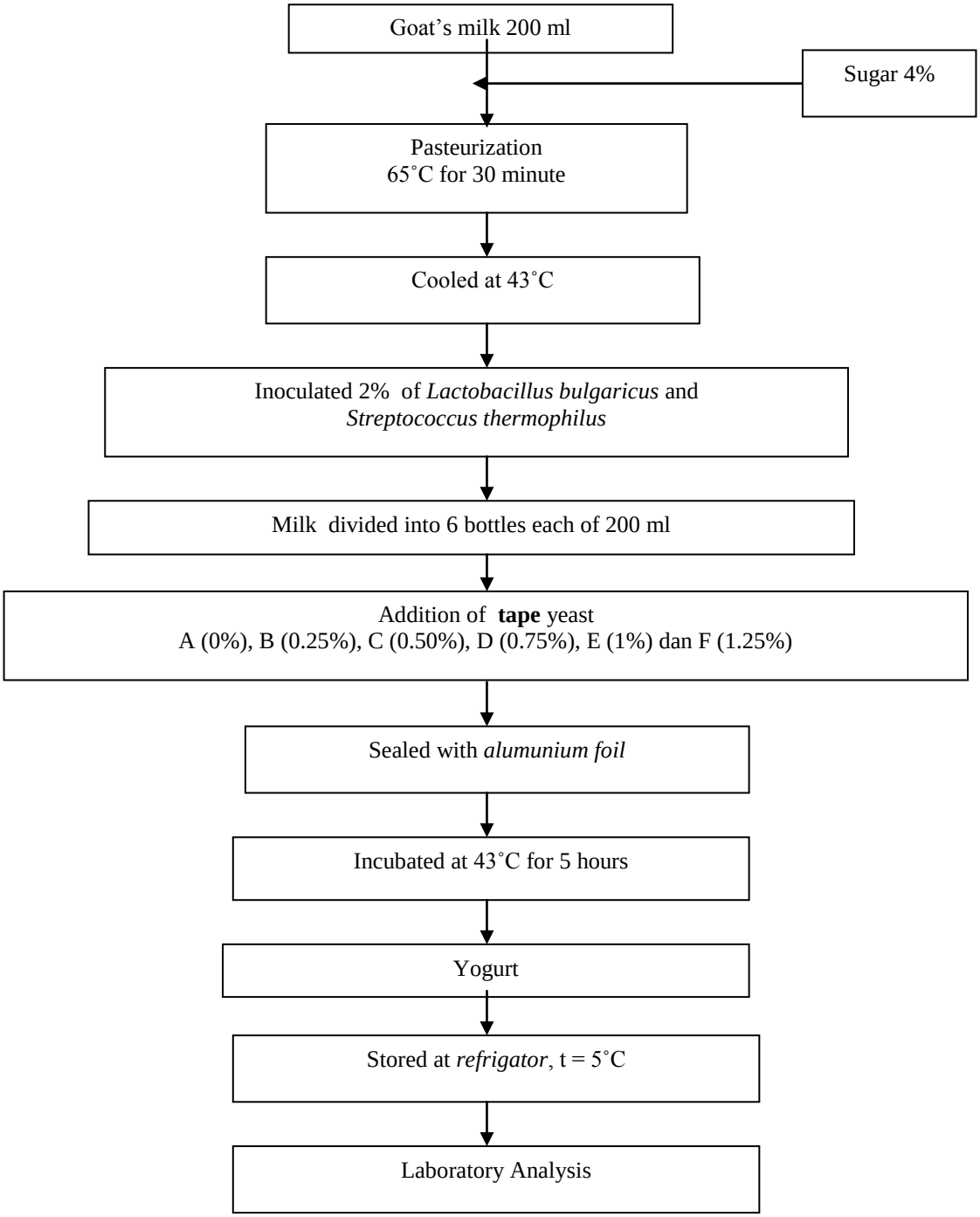


Fig 1. Flow Chart of Yogurt Making

Table 1. Moisture Content of Goat's Milk Yogurt

| Treatment | Moisture (%) |
|---|---|
| A | 83.21[a] |
| B | 82.32[b] |
| C | 81.00[c] |
| D | 80.53[d] |
| E | 80.05[e] |
| F | 79.87[f] |

*a.b : Mean with different superscript indicated a significant difference (P<0.01)*

Table 2. Acidity of Goat's Milk Yogurt

| Treatment | Acidity (% TTA) |
|---|---|
| A | 0.60[f] |
| B | 0.66[e] |
| C | 0.74[d] |
| D | 0.80[c] |
| E | 0.86[b] |
| F | 0.91[a] |

*a.b : Mean with different superscript indicated a significant difference (P<0.01)*

higher resulting protein coagulated followed by an increase in total solids of goat's milk yogurt.

This is stated by Widodo (2003), that at the pH is acid so protein in yogurt is coagulation and formation of curd gradually becoming more than common. Increased total solids of yogurt will be followed by a decrease in the moisture content of goat's milk yogurt so that the moisture content of goat's milk yogurt decreases. As proposed by Rahman et al. (1992) that the total solid will affect the moisture content of fermented milk products.

The highest moisture content of yogurt in treatment A becaused there were not tape yeast adding, so no yeast that can improve the activity of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* to modifying the lactose into lactic acid. As a result the lactic acid formed by yogurt bacteria is not so much. This was followed by the coagulation of proteins was low so that the total solids in goat's milk yogurt was less and goat's milk yogurt moisture content remains high at 83.21%.

*3.2. Acidity (%TTA)*

Statistical analysis showed that the addition of *Saccharomyces cerevisiae* in tape yeast has increased the acidity of goat's milk significantly (P <0.01), where the highest of acidity is in treatment F (0.91% TTA) followed by treatment E (0.86% TTA), D (0.80% TTA), C (0.74% TTA), B (0.66% TTA) and the lowest is in treatment A (0.60% TTA) as shown in Table 2.

Activity of yeast in the fermentation of milk will utilize glucose and galactose as modified of lactose by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* for modification into lactic acid so that the lactic acid production will increase.. In accordance with Fardiaz (1992) statement that the sugars which can be fermented by yeast are glucose, galactose, lactose, maltose. This is similarly with the Nurwantoro and Djarijah (1999) statement that the formation of lactic acid caused by modification of lactose into glucose and galactose by the enzyme lactase produced by microbes are then converted into specific types that is lactic acid. Albaari and Murti (2003) stated that the symbiosis between bacteria will accelerate the fermentation.

Symbiosis of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* will cause a low pH and acidity, equivalent with lactic acid which is higher than the single culture, so the acidity formed on goat's milk yogurt is higher than the yogurt without addition of tape yeast. As seen from this study results, the addition of tape yeast up to 1.25% on treatment F has produced goat's milk yogurt acidity highest that is 0.91% TTA.

The low content of lactic acid in the treatment A (0.60% TTA) caused of no added yeast, so no yeast that can improve the activity of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in modifying the

lactose into lactic acid. As a result of lactic acid formed is not a lot, so the acidity of the yogurt of goat's milk at treatment A is low. Lactic acid total has fulfilled SNI where the lactic acid total of yogurt is 0.5% - 2.0% TTA.

*3.3. Lactic Acid Bacteria Colony Count*

Statistical analysis indicated that the addition of *Saccharomyces cerevisiae* in tape yeast has increased lactic acid bacteria colony count of goat's milk yogurt (P <0.01). The lactic acid bacteria colony count of goat's milk yogurt in treatment F (9.53 x $10^9$ CFU / ml) were highest significantly (P <0.01) followed by treatment E (8.63x $10^9$CFU / ml), D (8.16 x $10^9$ CFU / ml), C (6.36 x $10^9$ CFU / ml), B (4.00 x $10^9$ CFU / ml) and A (3.33 x $10^9$ CFU/ml) as shown in Table 3.

Increased lactic acid bacteria colony count of goat's milk yogurt following with the addition of tape yeast caused of tape containing yeast which acts as a probiotic and immunostimulant which may improve the viability of yogurt like *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, so that lactic acid bacteria colony count of goat's milk yogurt will increase. In accordance with the Surono (2004) statement that the cooperation between the yeast and bacteria in the fermentation is mutual benefit, the yeast will produce compounds that stimulate the growth of lactic acid bacteria.

The higher the addition of yeast tape in goat's milk yogurt making it also increases the content of yeast acting as probiotics can stimulate the growth of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* so as to stimulating it's viability, thus the number of microorganisms increase. As shown in this study that the addition of tape yeast up to 1.25% (treatment F) resulted in total LAB colony count was highest of 9.53 x 109 CFU / ml.

Table 3. Lactic Acid Bacteria Colony Count of Goat's Milk Yogurt

| Treatment | LAB Colony Count (x$10^9$CFU.ml) |
|---|---|
| A | $3.33^{fe}$ |
| B | $4.00^{e}$ |
| C | $6.36^{d}$ |
| D | $8.16^{cb}$ |
| E | $8.63^{b}$ |
| F | $9.53^{a}$ |

*Note: a.b mean with different superscript indicated a significant difference (P<0.01)*

The low lactic acid bacteria colony count of goat's milk yogurt in treatment A (3.33 x $10^9$ CFU / ml) caused of no added yeast, so no yeast in goat's milk yogurt which acts as a probiotic to stimulate the growth of *Lactobacillus bulgaricus* and *Streptococcus thermophiles.* This causes the lactic acid bacteria colony count in goat's milk yogurt to be less. Consequently lactic acid bacteria grew only slightly than goat's milk yogurt is added tape yeast. If related with other variables such as moisture content and acidity, the lactic acid bacteria colony content is influenced by these variables as a result of the addition of tape yeast. The higher addition of yeast tape yeast on goat's milk yogurt will be followed by increased acidity, lowering the moisture and followed by increased of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* growth, so that will increase the lactic acid bacteria colony count.

## Conclusion

The addition of tape yeast was significant increasing acidity and lactic acid bacteria colony count significantly (P <0.01) and lowering the moisture content of goat's milk yogurt. The addition of tape yeast at 1.25% is the best in producing yogurt goat's milk yogurt.

## References

Albaarri dan Murti T. W. 2003. Analisa pH, keasaman, dan kadar laktosa pada yakult, yogurt, kefir. proceeding simposium nasional hasil-hasil

penelitian di Unika Soegijapranata, Semarang 22 Maret 2003 ISBN: 979-8366-53-0.

Badan Standardisasi Nasional. 2009. Standar Mutu Yogurt (SNI-01-2981-2009).

Fardiaz, S. 1992. Analisis Mikrobiologi Pangan. Penerbit PT. Gramedia Pustaka Utama, Jakarta.

Helferich, W. and D. Westoff. 1980. All About Yogurt. Prentice Hall, Inc., Englewood Cliffs, New Jersey.

Hidayat, N., M, C. Padaga dan S. Suhartini. 2006. Mikrobiologi Industri. Penerbit C.V Andi Offset, Yogyakarta.

Hotnida, C. H. S. 2003. Pengaruh penggunaan *probiotik Saccharomyces cerevisiae* dalam ransum mencit *(Mus muscullus*) terhadap produksi dan reproduksi. Departemen Ilmu nutrisi dan Makanan Ternak. Fakultas Peternakan Institut Pertanian, Bogor.

Nurwantoro dan A. S. Djarijah. 1999. Mikrobiologi Pangan Hewani-Nabati. Penerbit Kanisius, Jakarta.

Rahman, A., S. Fardias., W.P. Rahayu., Suliantri dan C.C Nurwitri. 1992. Teknologi Fermentasi Susu. Pusat Antar Universitas Pangan dan Gizi. Institut Pertanian Bogor

Sodiq dan Abidin. 2008. Meningkatkan Produksi Susu Kambing Peranakan Etawa. Agromedia Pustaka, Tanggerang.

Steel, R. G. D and J. H. Torie. 1995. Prinsip dan Prosedur Statistik. Suatu Pendekatan Biometrika. PT. Gramedia Pustaka Utama, Jakarta.

Surono, I. S. 2004. Probiotik Susu Fermentasi dan Kesehatan. PT. Tri Cipta Karya, Jakarta.

Tamime, A. Y and R .K. Robinson. 1989. Yogurt Science and Tecnology. Pergamon press, New York.

Widodo, 2003. Bioteknologi Industri Susu. Cetakan ke-1. Lacticia Press, Yogyakarta.

# Characteristics and Antimicrobial Activity of Lactic Acid Bacteria Isolated from Dadih of Agam Regency

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## Abstract

Dadih is a traditional food of West Sumatra Province that has differences of bacteria in any districts. This product give a good effect for health to consumers. This research was aimed to know the characteristics of lactic acid bacteria isolated of dadih from Agam regency and inhibition examination on pathogen bacteria. Observation of Lactic acid bacteria characteristic with macroscopic and microscopic. This research used dadih from Agam regency. Isolate of lactic acid bacteria was isolated from dadih and characteristics, gram stanning and biochemistry test for identification. On research had been isolation and found 17 isolates of Lactic Acid Bacteria with characteristics of cell in coccus and bacil, Gram positive (+), catalase negative and homofermentative. Antimicrobial activity of all isolates can inhibited the growth of pathogen bacteria (Escherichia coli O157, Staphylococcus aureus ATCC 25923dan Listeria monocytogenes) with different activity. Dadih has good potential as healthy food to consumed.

Keywords: Dadih, pathogen bacteria, antimicrobial activity

## 1. Introduction

In line with the growing number of research done, public awareness of the health and condition of the body to keep healthy. This can be seen from the large number of food products that have a high quality and level of food safety. Animal and vegetable products are a source of food that is consumed daily by the community. Animal products, such as meat and eggs as well as some very popular processed products of society, because it has a high nutritional value such as proteins. This product is also easily obtained over the market. Meat (beef, mutton, and poultry such as ducks) and processed products are now easily found in the market, price is affordable and very popular community such as jerky, beef rendang, beef rendang runtiah, sausages, Nuggets, corned beef and other products. This can be seen from the level of meat consumption society of West Sumatra as much 0.070 kg/capita/year [1], although still less than other countries.

Dadih is a typical regional foods of West Sumatra and processed through a natural fermentation process of buffalo milk in bamboo tubes by lactic acid-producing microorganisms that are present naturally in the milk of the water buffalo [2]. As for the areas in West Sumatra which can potentially produce dadih were Solok, Agam, Tanah Datar, Limapuluh Kota and Sijunjung Regency. This product provides excellent benefits for the health of consumers because it has a very good nutritional value, such as for the treatment of cholesterol, diarrhea, sore teeth and digestion.

Nutritional dadih varies, depending on the production area [3]. Not all bacteria can be used as probiotics. Bacteria that categorized probiotics must qualify among others, no pathogens, safely consumed and are able to

survive in the digestive tract. Lactic acid bacteria (BAL) contains the ' good ' bacteria in humans and livestock, which has been widely used as probiotics. BAL is a group of bacteria that are able to convert the carbohydrates (glucose) into lactic acid. The types of bacteria that includes BAL is the family Lactobacillaceae, that Lactobacillus and Weissella and Leuconostoc Streptococcaceae family, especially, Streptococcus and Pediococcus.

Lactic acid bacteria is bacteria that have status of GRAS (generally recognized as safe) and have a very important role in the fermentation of food or feed and as an extra starter in a controlled condition. The main product of BAL is the lactic acid generated in the low pH conditions. These bacteria are also capable of producing antimicrobial compounds, including hydrogen peroxide, carbon dioxide, diasetil, asetildehid, D-amino acid, an isomer of reuterin and bacteriosin [4]. Antimicrobial compounds produced these bacteria are able to inhibit the growth of pathogenic bacteria such as Bacillus substilis, Staphylococcus aureus, Escherichia coli O157, Listeria monocytogenes and Listeria inoqua. Listeria monocytogenes is one of the highly pathogenic bacteria are able to live in conditions of pH and concentration of acid salt reaches 10% [5,6]. Therefore, the isolation and identfikasi BALES from the dadih, then proceed with the meguji ability of the isolates LAB to inhibit the growth of pathogenic bacteria.

## 2. MaterialandMethods

*2.1. Procedures*

*The isolation of lactic acid bacteria [7]*

Isolation of BAL dadih used was 1 g, included in test tubes containing 9 ml of MRS Broth (dilution 1:10), dilution of 10-1. 100 µ L 10-1 dilution, inserted in the tube contains 900 µ l MRS Broth dilution 10-2-10-7. Then, 100 µ L of the serial dilution of l0-7 diinokulasikan on the media MRS Agar with the method of spread, put in an anaerobic jar, incubated for 48 hours with a temperature 37oC. A single colony that characterize LAB.

*Identification of lactic acid bacteria [7]*

Identification of LABdadih samples done by 1 g put in a tube containing 9 ml MRS Broth (dilution 1:10), dilution of 10-1. Incubated in anaerobic conditions for 24 hours. After that, 100 µ L 10-1 dilution is inserted in the tube contains 900 µ l MRS Broth dilution 10-2, done to a dilution of 10-8. Then, 100 µ L of the serial dilution of l0-8 inuculated on the media MRS Agar with the method of spread, put in an anaerobic jar, incubated for 48 hours at 37oC. A single colony that characterize LAB (round slippery white yellowish) transferred to the media in order to MRS. pemumian the colony with the method of streak and incubated.

## 3. Result andDiscussion

*3.1.Number of Colony Lactic Acid Bacteria*

Number of colony lactic acid bacteria of dadih obtained in the study were calculated with CFU/g as 4 x 1010 CFU/g. Number of colonies LAB of dadih from Agam in accordance with criteria of FAO/WHO [8] because as a probiotic food LAB produced is at number $10^6$ – $10^8$ CFU/g. Number of colonies LAB of dadih because lactic acid bacteria in a bamboo tube used has an active role while the formation of the dadih. Number of colonies LAB as shown in Fig. 1.

These results are higher than [9] research that gets number of colonies LAB of dadihfrom Nagari Air Dingin, Solok as 1.48 x $10^8$ CFU/g. This is due, the process of making dadih are different in each region.

*3.2.Identification of Lactic Acid Bacteria*

Identification is done in two ways i.e. macroscopic and microscopic. Macroscopic observation (shape and color) of lactic acid bacteria (LAB) in this study found a colony of white-yellowish and spherical slick on the media MRS in order. Result of this research such as the following in Fig. 2.

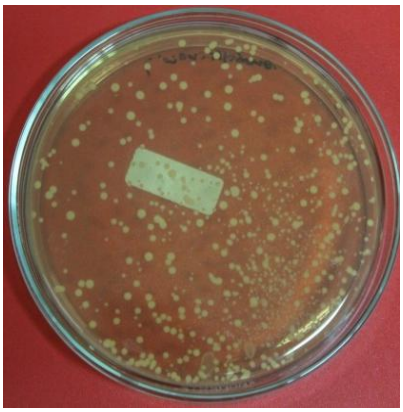


Fig.1. Number of colony lactic acid bacteria of dadih from agam regency

The chosen colony is a colony that is still a young ± 18 hours. The goal is to make the bacteria is not too old to be tested, since [10]who explained if the age of the bacteria too old, then the bacteria will tend to absorb safranin (red) so will be declared negative gram (-) although the bacteria are Gram-positive bacteria. The number of isolates found were 17 isolates and is a Gram positive bacteria (+) as shown in Figure 4 above. It is characterized by bacteria that absorb the color purple of crystal violet and has the shape of the stem (bacil). This is in accordance with statement of [10] stating that the Gram-positive bacteria will take on the color of the crystal violet purple despite being washed with alcohol and when given the safranin is red, the bacteria will still be purple, whereas red indicates gram-negative bacteria. [11] added that isolates the alleged Lactobacillus with meet the criteria have negative, catalase positive, Gram staining, nonmotil, and the shape of bacil.

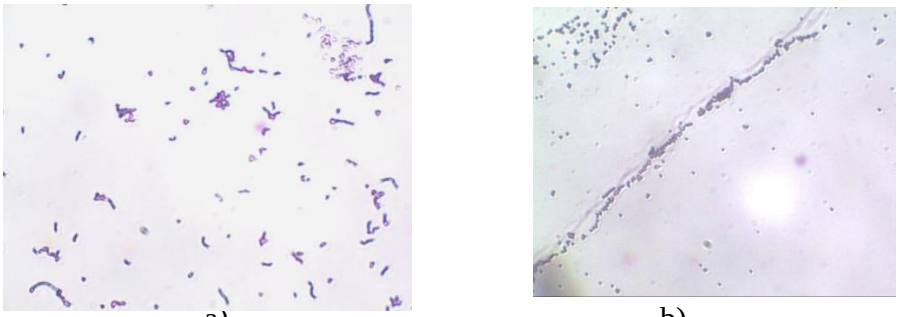
The difference in absorption of color is caused by the difference in membrane permeability and peptidoglycan gram positive organisms by gram negative, where permeability membrane Gram-positive organisms have Peptidoglycan cell walls thick enough than gram-negative, Gram-positive organisms have a cell wall thick enough (20-80 nm) and consists of 60 to 100% of Peptidoglycan. Cell walls are compact and less permiabel so that at the time of the granting of crystal violet, then the color of the substance enters the cell wall and at the time of washing with alcohol, the color purple has been tied to those unable to get out again so the colors can no longer safranin dye Gram-positive bacteria. Unlike the gram negative cell wall, cell wall contains less Peptidoglycan (10 to 20 percent), less compact and more permiabel. At the time of the giving of the crystal violet purple, then the color of the substance will dissolve upon washing with alcohol, and at the time of the giving of the color substance that then safranin coloring gram-negative [10].

*3.3.Antimicrobial Resistance Testing*

Antimicrobial resistance test observations on each test bacteria (Listeria monocytogenes, Staphylococcus aureus and Escherichia coli O157) as in Table 1.

Based on the above table and figure, proving that dadih can be used as a probiotic biosuplement may decrease the growth of pathogenic bacteria such as *Listeria monocytogenes, S. aureus* ATCC 25923, and *E. coli* $O_{157}$. Dadihfrom Agam Regency fermented past 48 hours is capable to inhibit bacterial third test (pathogen) with different diameters. The diameter of the inhibition zones visible on the widest against bacteria *Listeria monocytogenes*, *Escherichia coli* O157 and the smallest on *Staphylococcus aureus*. This is also conform with research [12] which States that, at 48 hours of prolonged storage the amount of lactic acid bacteria grow because bacteria grow properly acid-forming without any rivals, while it also pathogenic bacteria cannot live because it does not hold the acid and lactic acid bacteria can inhibit the growth of pathogenic bacteria.

The results of this research contrasts with [13] that get the highest inhibition zones against *Listeria* with different temperatures is 10 mm. [14] are conducting research using VCO-Lactobacillus casei BAL origin gives a



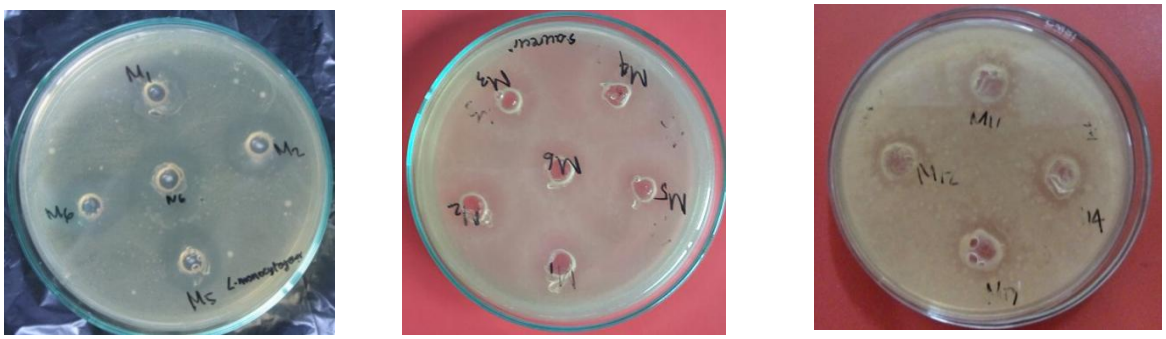
a) b)

Fig 2. a) *bacil* dan b) *coccus*

Table 1. Diameter of Inhibition Zone (mm)

| Isolates | Inhibition Zone (mm) | | |
|---|---|---|---|
| | *Escherichia coli* O157 | *Staphylococcus aureus* | *Listeria monocytogenes* |
| M1 | + | ++ | ++ |
| M2 | + | ++ | ++ |
| M3 | + | ++ | + |
| M4 | + | + | ++ |
| M5 | + | ++ | ++ |
| M6 | + | ++ | ++ |
| M7 | + | + | +++ |
| M8 | + | + | +++ |
| M9 | + | ++ | ++ |
| M10 | + | + | +++ |
| M11 | + | + | ++ |
| M12 | + | + | +++ |
| M13 | + | + | ++ |
| M14 | + | + | +++ |
| N3 | + | + | +++ |
| N5 | + | + | +++ |
| N6 | + | + | +++ |

Information : '+' = 12-17 mm, '++' = 18-23 mm and '+++' = >23 mm



*a. Listeria monocytogenes* *b. Staphylococcus aureus* *c. Escherichia coli* O157

Fig. 3. Inhibition Zone (mm)

real influence ($P < 0.05$) on the diameter of the inhibition zones against test bacteria Escherichia coli with a diameter of 6.45 ± 0.50 mm. Higher also than research [15] that get the highest zone of LAB of dadih Palupuh inhibition against the bacteria *Staphylococcus aureus* with diameters 8-14 mm and [16] also get the inhibition zones against *Staphylococcus aureus Salmonella thypii,* and *Escherichia coli* with diameter 8-14 mm.

## Conclusion

The conclusions of this research are the isolates found in the dadih is LAB with rounded, slippery, yellowish white creature with the total number of colonies 4 x $10^{10}$ CFU/g. Gram positive (+), form of bacil and cocci. LAB obtained isolates were able to inhibit the growth of pathogenic bacteria *Escherichia coli* O157, *Listeria monocytogenes* and *Staphylococcus aureus* with the biggest zone is *L. monocytogenes.*

## References


[1] Animal Husbandry Department of West Sumatera. 2012. Animal Husbandry Database of West Sumatera Province of 2002-2011. Animal Husbandry of West Sumatera, Padang.

[2] Purwati, E., Arif and A. Rahmadi. 2011. Textbook Technology of Dadih. Scholar, Bogor.

[3] Usmiati, S. and Risfaheri, 2012. Improvement of Dadih as an Indigenous Probiotic Functional Food of West Sumatera. J. Litbang Pert. Vol. 32 No. 1. MAret 2013: 20-29.

[4] Cintas LM, Casaus MP, Herranz C, Nes IF, Hernández PE (2001) Review: Bacteriocins of Lactic Acid Bacteria. Food Sci Tech Int 7:281–305.

[5] Tasara T, Stephan R. 2006. Review cold stress tolerance of Listeria monocytogenes: A review of molecular adaptive mechanisms and food safety implications. J Food Protection 69:1473–1484.

[6] Vescovo M, Orsi C, Scolari G, Torriani S (1995) Inhibitory effect of selected lactic acid bacteria on microflora associated with ready-to-use vegetables. Letters in Applied Microbiol 21:121–125

[7] Purwati, E., S. Syukur and Hidayat. 2005. Lactobacillus sp. Isolation from Biovicophitomega as a probiotic. In The Indonesia Institute Of Science Proceedings.

[8] FAO/WHO. 2002. The Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food. London.

[9] Purwati, E., S. Syukur, Husmaini, H. Purwanto and R.P. Pasaribu. 2014. Molecular characterization of lactic acid bacteria isolate dadih Solok Regency Air Dingin of West Sumatera. Vol. 40, no. 2, February 15, 2014:134-146.

[10] Unus, U. 2005. Basic Microbiology. Publisher Papas Sinanti Rays, Jakarta.

[11] Sunaryanto, R. and B. Marwoto. 2012. The isolation, identification and characterization of lactic acid bacteria from dadih milk of water buffaloes. Indonesia science and technology Journal Vol. 14, no. 3, December 2012, pp. 228-233.

[12] Yang, E., L. Fan, Y. Jiang, C. Doucette and S. Fillmore. 2012. Antimicrobial activity of Bacteriocin producing lactic acid bacteria – isolated from cheeses and yogurts. AMB Express 2012, 2:48. Http://www.amb-express.com/content/2/1/48.

[13] Rahmadi, A., I. Abdiah, M. D. Sukarno and T. Purnaningsih. 2013. Physicochemical and antibacterial characteristics of virgin coconut oil fermented with lactic acid bacteria. J. Tech. and Food Industry. Vol. 24 No. 2 2013. ISSN: 1979-7788.

[14] Depson, R.. Molecular identification and influence the granting of potential probiotic lactic acid bacteria (LAB) from dadih against cholesterol meat duck genetic resources of West Sumatra. Thesis. Andalas University Graduate. Andalas University, Padang.

[15] Trisna, N. W.. Molecular identification and influence the granting of probiotic lactic acid bacteria (BAL) from dadih from sijunjung against cholesterol levels in meat duck pitalah genetic resources in West Sumatra. Thesis. Andalas University Graduate. UNAND, Padang.

# A Comparative Study on Composition and Microbiological of Buffalo Milk From Different Location in West Sumatra

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## Abstract

The aim of this research is to compare the composition and the number of lactic acid bacteria of buffalo milk from a different location in West Sumatra (50 Kota, Agam, Tanah Datar and Solok), Indonesia. The Milk samples were collected in a sterile bottle and store at 4$^{o}$C until was carried out in the laboratory. The composition of buffalo milk was analyzed, including pH, spescific gravity, water, fat, and protein. The composition of buffalo milk was recorded that 80.48% ± 1,12 - 81.03% ± 1.91water, 7.18% ± 0.09 -7.83% ± 0.98 fat,6.22% ± 0.39 - 7.83% ± 0.19 protein, 6.06 ± 0.02 - 6.39 ± 0.25 pH, 1.028 ± 0.001 - 1.030 ± 0.001specific gravity and the number of lactic acid bacteria $3 \times 10^6 – 257 \times 10^6$ CFU/g. There are non-significantly different of the nutrition Buffalo milk from West Sumatra. Buffalo milk from Agam and Tanah Datar location has a high as potential source of lactic acid bacteria.

Keywords: Buffalo milk, composition, West Sumatra, lactic acid bacteria

## 1. Introduction

Buffalo milk is milk produced from the domesticated water buffalo (Bubalus bubalis). Buffalo milk is different from other ruminant milk because it contains fat and higher protein. High levels of fat, protein, and other dissolved solids made of buffalo milk is more easily processed into dairy products such as cheese, yogurt and ice cream. The high demand for products from buffalo milk due to higher sensory quality.

Buffalo milk has nutritional composition is very complex. The composition of buffalo milk fat-containing 84.25 g / kg, solids nonfat 94.80 g / kg, protein 39.68 g / Kg, lactose 48 g / kg, ash content of 7.13 g / Kg, the acidity of 0164%, the water content of 826.60 g / kg and pH 6.37 [1]. High levels of lactose in milk buffalo buffalo milk makes the potential for growth of lactic acid bacteria. According to [2], buffalo milk and yogurt made from buffalo milk has better nutritional value than cow's milk protein, carbohydrates and calcium. In addition, buffalo milk is also a source of linoleic acid. The role of linoleic acid which are potentially as anti carsinogenic and anti dipogenic. According to [3], who reported that a child who is allergic to cow's milk is able to tolerate milk buffalo.

In West Sumatra buffalo milk is the main ingredient in processing Dadih. Dadih is fermented milk of buffalo from West Sumatra. Dadih is made by fermenting naturally from buffalo milk is incorporated directly into bamboo tubes and fermented for 2 × 24 at room temperature. Average production of buffalo milk in one day is between 1-2 liters / buffalo. Statistical data out 2010-2013 showed buffalo milk production in West Sumatra are 50Kota, Agam, Tanah Datar and Solok, each of which is 137 tons, 200 tons, 114 tons and 97 tons.

Various types of microorganisms are yeasts, molds and bacteria contained in fresh milk. But among types of bacteria, only lactic

acid bacteria that have the ability to produce lactic acid from milk sugar through fermentation process so that the lactic acid bacteria can be regarded as the most dominant bacteria in fresh milk. Lactic acid bacteria (LAB) is a gram-positive, non-spore, spherical or rod, which produces lactic acid as an end product of fermentation of carbohydrates [4]. Activity of lactic acid bacteria can cause a decrease in pH, which pathogenic bacteria can not grow at low pH. Lactic acid bacteria are also classified as probiotic bacteria that have antimicrobial activity against some specific microorganisms, tolerant of stomach acid and is not dangerous [5]-[6].

Based on research[7], explains that the lactic acid bacteria present in cow's milk, goat's milk, sheep's milk, camel's milk and buffalo milk that comes from the region of Gwalior, Madhya Pradesh, India. From his research found five types of bacteria from each source of milk, namely *Streptococcus thermophillus* from goat's milk, *Lactococcus lactis* from buffalo milk, *Streptococcus gallolyticus* from camel milk, *Streptococcus thermophilus* from cow's milk and *Lactobacillus delbrueckii* from sheep's milk were identified as having the ability to produce acid lactate. In buffalo milk contained lactic acid bacteria is *Lactococcuslactis*, which has the ability to produce lactic acid, which is 57.61%.

This study aimed to compare the composition of buffalo milk and total lactic acid bacteria contained in four districts in West Sumatra, Indonesia.

## 2. Material and Methods

*2.1.Material*

This research material is buffalo milk obtained from four location in West Sumatra, namely 50 Kota, Agam, flat ground and Solok. The materials used in this study is the Man Rogossa Sharpe Agar (MRSA) (Merck), MRS Broth (Merck), DeriPro DNA extraction, Muellier Hinton Agar (MHA) (Merck), listeria broth (Merck), Buffered Peptone Water (Biolife) , glycerol, safranin (R & M Chemicals), agarose, crystal violet (R & M Chemicals), safranin (R & M Chemicals), distilled water, alcohol, spritus, HCl 0,1N NaOH 0,1N, bacteria *Listeria monocytogenes.*

The equipment used is PCR, gel electrophoresis, cool box, laminar fow, anaerobic jar, thermometer, pH meter, a petri dish, a loopful, incubator (Infors HT-ECOTRON), measuring cups, analytical balance, erlenmeyer, Bunsen lamp, test tubes , measuring cups, cootton buds, glass beaker, a pipette, hockey stick, autoclave, Centrifuge 5417R, vortex, spectrophotometer UV-1800 (Shimidzu) and a micro pipette. This study uses laboratory of Animal Processing Technology and Biotechnology Laboratory of the Faculty of Animal Science Andalas University.

*2.2.Methods*

*2.2.2. Buffalo Milk Quality Measurement*

Buffalo milk quality measured in this study is a composition of water, protein, fat, pH and density.

*2.2.3. Total Lactic Acid Bacteria Colonies*

The steps taken in calculating the total colonies of lactic acid bacteria (LAB) according to [14] can be explained as follows:All the necessary equipment such as a petri dish (petridish), test tubes, Erlenmeyer, eppendorf tubes, micro pipette tip, hockey stick, sterilized in an autoclave at 121 ° C for 15 min with a pressure of 15 lbs.Prepared media was prepared by dissolving 16 443 g de Mann ROGOSA Sharpe (MRS) broth (Merck) in 315 ml of distilled water for 7 samples and to dilution 10-5 (Creation in general is 52,2g MRS Broth in 1000 ml of distilled water). Furthermore homogenized with a magnetic stirrer on a hot - plate at a temperature of 100 ° C, then in the autoclave (15 minutes, 121 ° C and a pressure of 15lbs). Prepared media de Mann ROGOSA Sharpe (MRS) Agar (Merck) by dissolving 6,951 g

MRS order in 105 ml of distilled water (Creation in general is 66.2 g MRS order in 1000 ml distilled water), then homogenized with a magnetic stirrer, above hot plate at a temperature of 100 ° C, and in the autoclave, after a rather cold (± 55 ° C) and then poured into a petri dish respectively 7 -masing as much as ± 15 ml. Using a spoon sterile and aluminum foil sample weighed as much as 1 g, then diluted with 9 ml de Mann ROGOSA Sharpe (MRS) Brothroth, then divortex until homogeneous. This result is called dilution 10-1.The result of the dilution taken 1 ml put in a test tube containing 9 ml de Mann ROGOSA Sharpe (MRS) broth, then divortex until homogeneous. After serial dilution, sample is taken da planted by the method that has been spread on petridish containing MRS media order then leveled with a hockey stick that had previously been given alcohol and burned with Bunsen. This work was done in lamina flow and near Bunsen.The inoculum is stored in an anaerobic jar and put in the incubator for 24 hours at 37 ° C. For 24 hours colonies growing BAL viewed by using the tools of Quebec colony counter. The calculation result is multiplied by ten colonies BAL.

## 3. Result andDiscussion

*3.1. The Quality of Buffalo Milk*

High levels of fat, protein, and other dissolved solids made of buffalo milk is more easily processed into dairy products such as cheese, yogurt and ice cream. The high demand for products from buffalo milk due to higher sensory quality. While in West Sumatra buffalo milk processed naturally be called fermented milk curd.

Buffalo milk has nutritional composition is very complex. Table 1 shows the water content of 80.48% ± 1.12 - 81.03 ± 1.91%, fat 7.18±0.09 - 7.88% ± 0.98, protein, 7.22 ± 0.39 – 0.19 ± 7.83%, pH 6.06 ± 0:02 to 6.39 ± 0.25, spesific gravity (BJ) 1,028 ± 0001-1030 ± 0.001 buffalo milk. While research (Khan et al., 2007), the composition of buffalo milk fat 84.25%, solids non-fat 9,480%, protein 3,968%, lactose 4.8%, ash content of 0.713%, acidity 0.164%, water content 82.660% and pH 6:37. High levels of lactose in milk buffalo buffalo milk makes the potential for growth of lactic acid bacteria. (NANDA and Nakao, 2003), reported the swamp buffalo milk, has a moisture content of 81%, fat content (9-15%), protein (7.1%), and lactose (4.9%). Dairy buffalo (Murrah Breed of Bubalusbubalis) of the Cantal region, France, has a pH of 6.81, fat 70 g / Kg, lactose 52.1 g / kg, ash 8.4 g / Kg, and some of the minerals calcium, magnesium, phosphorus, sodium, Potassium [5]. [8], examined the physicochemical properties of buffalo milk from Gujrat, Pakistan. Buffalo milk containing 7.97% fat,% protein 4:36, 5:41 lakatosa%, ash 0.81% and pH 6.7. [9], examines the composition of buffalo milk from several countries, have an average fat content of 6.6-8.8 g / 100g, lactose 4.5 - 5.2 g / 100 g, protein 3.8 - 4.5 g / 100g,

According to [2], buffalo milk and yogurt made from buffalo milk has better nutritional value than cow's milk protein, carbohydrates and calcium. In addition, buffalo milk is also a source of linoleic acid. The role of linoleic acid which are potentially as antikarsibogenik and antidipogenic. Added by [5], the pH of buffalo milk from Islamabad is 6.7.

Milk contains nutrients that are very complete, so it can support the growth of microorganisms which are very complex that can come from various sources, and milk themselves. The role of microorganisms, among others in the process of fermentation of dairy products, causes of decay, supporting health and cause disease. [10].

Table 1. The Composition of Buffalo Milk

| Location | Nutrition | | | | |
|---|---|---|---|---|---|
| | Water | Fat | Protein | pH | Density |
| 50 Kota | 80.48 ± 0.93 | 7.34 ± 0.50 | 7.57 ± 0.21 | 6.39 ± 0.25 | 1.028 ± 0.001 |
| Agam | 81.44 ± 1.17 | 7.18 ± 0.09 | 7.22 ± 0.39 | 6.06 ± 0.04 | 1.028 ± 0.001 |
| Tanah Datar | 81.03 ± 1.91 | 7.88 ± 0.98 | 7.83 ± 0.19 | 6.08 ± 0.03 | 1.028 ± 0.001 |
| Solok | 80.48 ± 1,12 | 7.73 ± 0.22 | 7.23 ± 0.76 | 6.10 ± 0.02 | 1.028 ± 0.001 |

Table 2.The Number of Lactic Acid Bacteria in Buffalo Milk

| Location | The Number of BAL($1 \times 10^6$ CFU/g) |
|---|---|
| 50 Kota | 3 |
| Agam | 190 |
| Tanah Datar | 257 |
| Solok | 24 |

*3.2.The Number of Lactic Acid Bacteria*

Tabel 2 showed the number of lactic acid bacteria in buffalo milk $3 \times 10^6$ CFU/g. Total lactic acid bacteria buffalo milk that comes from the Bulgarian Murrah buffalo 3.22 x $10^5$ /$cm^3$[11] Lactic acid bacteria isolated from buffalo milk by [12], namely *Lactobacillus acidophilus, L. delbrueckii ssp. Bulgaricus, Lactococcuslactis ssp. Cremoris,L. lactisssplactis* and *Streptococcus thermophilus.*

Furthermore, [7]reported that the lactic acid bacteria present in cow's milk, goat's milk, sheep milk, camel milk and buffalo milk that comes from the region of Gwalior, Madhya Pradesh, India which each is the *Streptococcus thermophilus* from goat's milk, buffalo milk *Lactococcuslactis, Streptococcus gallolyticus*from camel's milk, cow's milk *Streptococcus thermophilus* and *Lactobacillus of delbrueckii* from sheep's milk.

In buffalo milk contained lactic acid bacteria is *Lactococcuslactis*, which has the ability to produce lactic acid, which is 57.61%. Subsequently [13]found that lactic acid bacteria found in buffalo milk that comes from North Sumatra, Gram-positive, rod shape and round shape is *Lactobacillus plantarum, L. brevis, L. L. paracaseipentosus*and *Lactococcuslactis.*

## Conclusion

The results of proximate analysis buffalo milk from West Sumatra showed the water content of 80.48% ± 1.12 - 81.03 ± 1.91%, ±% fat 7.18 0:09 - 7.88% ± 0.98,% protein 7:22 ± 0:39 - 0:19 ± 7.83%, ± pH 6:06 0:02 to 6:39 ± 0:25, density (BJ) 1,028 ± 0001-1030 ± 0.001 and total lactic acid bacteria 3-257 × 106 CFU / g. Total lactic acid bacteria highest buffalo milk comes from the district of Agam and Tanah Datar.

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## References

1. M. A. S. Khan, M. N. Islam, and M. S. R. Siddiki, "Physical and chemical composition of swamp and water buffalo milk: a comparative study," *Ital. J. Anim. Sci.*, vol. 6, no. sup2, pp. 1067–1070, 2007.
2. X. Han, F. L. Lee, L. Zhang, and M. R. Guo, "Chemical composition of water buffalo milk and its low-fat symbiotic yogurt development," *Funct. Foods Health Dis.*, vol. 2, no. 4, pp. 86–106, 2012.

3. W. J. Sheehan and W. Phipatanakul, "Tolerance to water buffalo milk in a child with cow milk allergy," *Ann. Allergy Asthma Immunol. Off. Publ. Am. Coll. Allergy Asthma Immunol.*, vol. 102, no. 4, p. 349, 2009.
4. E. Isolauri, S. Salminen, and A. C. Ouwehand, "Probiotics," *Best Pract. Res. Clin. Gastroenterol.*, vol. 18, no. 2, pp. 299–313, 2004.
5. R. Z. Ahmed, K. Siddiqui, M. Arman, and N. Ahmed, "Characterization of high molecular weight dextran produced by Weissella cibaria CMGDEX3," *Carbohydr. Polym.*, vol. 90, no. 1, pp. 441–446, 2012.
6. N. Nordin, F. Abu Bakar, and M. AS, "Antibacterial agents produced by lactic acid bacteria isolated from Threadfin Salmon and Grass Shrimp," *Int. Food Res. J.*, vol. 20, no. 1, pp. 117–124, 2013.
7. R. Sharma *et al.*, "Characterization of lactic acid bacteria from raw milk samples of cow, goat, sheep, camel and buffalo with special elucidation to lactic acid production," *Br. Microbiol. Res. J.*, vol. 3, no. 4, p. 743, 2013.
8. A. Mahmood, S. Usman, and others, "A comparative study on the physicochemical parameters of milk samples collected from buffalo, cow, goat and sheep of Gujrat, Pakistan," *Pak. J. Nutr.*, vol. 9, no. 12, pp. 1192–1197, 2010.
9. M. H. A. El-Salam and S. El-Shibiny, "A comprehensive review on the composition and properties of buffalo milk," *Dairy Sci. Technol.*, vol. 91, no. 6, pp. 663–699, 2011.
10. L. Quigley *et al.*, "The complex microbiota of raw milk," *FEMS Microbiol. Rev.*, vol. 37, no. 5, pp. 664–698, 2013.
11. S. Boycheva, T. Dimitrov, and T. Iliev, "Investigation on microflora of buffalo milk," *Bulg. J. Agric. Sci.*, 2002.
12. T. Aziz, H. Khan, S. M. Bakhtair, M. Naurin, and others, "Incidence and relative abundance of lactic acid bacteria in raw milk of buffalo, cow and sheep," *Anim. Plant Sci.*, vol. 19, no. 4, pp. 168–173, 2009.
13. H. Rizqiati, C. Sumantri, R. R. Noor, E. Damayanthi, and E. I. Rianti, "Isolation and identification of indigenous lactic acid bacteria from North Sumatra river buffalo milk," *Indones. J. Anim. Vet. Sci.*, vol. 20, no. 2, p. p–87, 2015.
14. Syukur, S., L.S. Utami., E. Purwati, UrnenrnidanJamsari, 2011. Screening and In vitro Antimikrobial, Protease activities From Lactic acid Bacteria Associated With Green Cacao Fermentation in West Sumatera, Indonesia. Prosiding Seminar Internasional HKI, Pekanbaru.

# Effect of Addition White Oyster Mushroom (*Pleurotus ostreatus*) and Carrot (*Daucus carota L*) In Probiotic Duck Nugget On Protein, Calcium and Organoleptic Value

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## Abstract

This research was aimed to determine the effect of interaction between white oyster mushroom (*Pleurotus ostreatus*) and carrot (*Daucus carota L*) on protein, calcium and organoleptic value in probiotic duck nugget. The method used in this study was experimental method by using a randomized block 3 x 3 factorial design with two replications as a group. The factor A consisted of white oyster mushroom, namely A1: 0%, A2: 10%, A3: 20%, and the factor B consisted of carrot, namely B1: 0%, B2: 10%, B3: 20%. The parameters measured were the protein, calcium and organoleptic value in probiotic duck nugget. The results showed there was a significant interaction ($P<0.05$) between the white oyster mushroom and carrot on calcium value, Whereas on protein and organoleptic value did not show significant interaction ($P>0.05$). The best percentage of addition for probiotic duck nugget that result from this researh are 10% of white oyster mushroom and 20% of carrot, with value of protein 15,06%, calcium 1,14% (114.24 mg/100 g) and organoleptic that consisted of color 2,04, aroma 1,76, texture 1,72 and flavor 1,96.

Keywords: probiotic duck nugget, white oyster mushroom, carrot, protein, calcium and organoleptic value

## 1. Introduction

Poultry meat is now starting to get the attention of the public. Demand for poultry meat higher and higher to get enough meat consumption. One poultry meat producer is a duck. Duck meat can contribute a source of protein for the human body. Protein source from outside the body is best of animal protein, but the sources of animal protein in general have high cholesterol levels.

Drake produce meat is low in cholesterol, so healthy to eat all ages of consumers. It is supported by Setiabudi (2011) in Trisna (2012) states the cholesterol content of duck meat reaches 50 mg / dl, whereas in the previous study on duck meat Sikumbang Jonti with supplemental feeding of probiotics cholesterol only 25.65 mg / dl. Probiotics duck meat are healthier in terms of nutritional content, compared with ordinary duck meat therefore this study using probiotics duck meat as a raw material for making nuggets.

Food diversification duck meat fast food such as meat nuggets into innovation where the texture of the meat becomes more tender and easier to serve. Nugget is known as a fast-food products *(fast food)* with the meat quality is not too good. Selection of probiotic duck meat because the meat is expected to be more healthy, due to consumption of probiotics by ducks during maintenance.

Nugget with the addition of white oyster mushroom *(Pleurotus ostreatus)* as a source of fiber and calcium, which can increase calcium levels nugget.

Carrots *(Dancus carota L)* as an antioxidant to contain beta-carotene which can prevent the oxidation of LDL *(Low Densinty Lipoprotein)* so that lowering cholesterol levels. Interaction between white oyster mushrooms and carrots are expected to improve the nutritional content of foods such as increased levels of protein and calcium. White oyster mushroom *(Pleurotus ostreatus)* can be a source of protein, calcium and fiber in the form of polisakrida.The purpose of this study was to determine the optimal addition of white mushroom *(Pleurotus ostreatus)* and carrots *(Daucus carota L)* at the nugget ducks probiotics.

The usefulness of this study is to provide information and knowledge to the community that the addition of white oyster mushroom *(Pleurotus ostreatus)* and carrots *(Daucus carota L)*that can improve the nutritional content nugget. Information from this research can be a reference to how the handling and processing of white oyster mushroom *(Pleurotus ostreatus)* and carrot *(Daucus carota L)* for additional comestible nugget.

The hypothesis of this study is the interaction between the addition of white oyster mushroom *(Pleurotus ostreatus)* and carrots *(Daucus carota L)* at the nugget ducks probiotics to increased levels of protein, calcium and probiotics organoleptic duck nuggets.

## 2. Material and Methods

*2.1.Place and Time Research*

This research was conducted at the Laboratory of Livestock Product Technology Faculty of Animal Husbandry Andalas UniversityPadang ranging from 1 February 2015 until March 28, 2015.

*2.2.Tools and Materials*

The tools used in this study is a tool grinder meat, stove, cutting boards, spoons, pots, knives, test tubes, erlemeyer 500 ml and a stir bar. Tool to calculate the protein content: Kjeldhal flask, funnel, distillation flask, tool refiners, 250 ml beaker, mumps pipette 25 ml, 500 ml flask. Tool to calculate the calcium is *Atomatic Absrotion Spectrophotometer*(AAS), flask and furnaces. Tool to calculate the organoleptic value is oven, stationery, a presentation of the sample, glass.

*2.3.Research Procedure*

Research procedures for the manufacturing process steps nugget is the first probiotic duck meat separated from the bones weighing 500g, prepare the dough. White oyster mushrooms and carrots that had been weighed and then separate and mixed into the meat along with the dough in accordance with the treatment. The dough nuggets ducks probiotics after mixed (according to treatment) in print and then steamed for 30 minutes. Powdery with panir process is performed after cold nugget of steamed, then packed in vacuum plastic and stored freezer for 16 hours

2.4 *Testing and Data Analysis*

Tests performed on samples of probiotic duck nuggets that levels of protein, calcium and organoleptic value. The data was based on the experimental design. The data analysis is required to determine the difference between treatments in the research. Analysis of the data in this study using a diversity analysis ANOVA (Analysis of Variance) with 95% confidence interval that will produce variants. If the calculated F is less than F table then there is no interaction between treatments. If the F count more than F table then no interaction or significantly different between treatments. If there is a real difference in the interaction of both treat do

DMRT (Duncan's Multiple Range Test) with a confidence interval ($\alpha = 0.05$).

## 3. Result and Discussion

*Analysis of Protein Levels Probiotics Nugget Ducks*

Table 1 shows the average protein content nugget ducks probiotics with the provision of 0%, 10% and 20% of white oyster mushrooms and carrots ranged between 13.05% - 16.46% with the average protein content nugget duck probiotics highest 16:46% in A1B1 and the lowest was 13.05% on A3B3. Results of analysis of variance showed no interaction (P> 0.05) factor A (oyster mushroom) with factor B (grated carrots) the protein content of the probiotic duck nuggets.

The absence of these interactions caused by protein content in carrots is lower than the white oyster mushrooms and meat, but the analysis of variance showed the effect of significantly different (P <0.05) on each factor.

The protein content of duck nuggets probiotic treatment with factor A (oyster mushroom) and factor B (carrots) can not pass the protein content of meat nuggets. This is due to the white oyster mushroom lower protein content than meat where white mushrooms protein content is 10.5% and protein content of carrots that were not as white oyster mushroom or meat which is about 0.93%, so that did not show an interaction between treatments. Protein oyster mushroom is a globular protein, globular protein is a protein that is damaged in processing. This protein has a same type of characteristics that is easily denatured.

A factor treatment (oyster mushroom) and factor B (carrots) as a source of vegetable protein can not Substituting animal protein. This is due to the best vegetable proteins found in grains, while the white oyster mushroom is a kind of mushroom and carrots included in the tubers. It is supported by Soedarmo in Rokhmah (2008) suggested that high-value vegetable protein are nuts and seeds.

Analysis showed the diversity of the treatment effect of each factor were significantly different (P <0.05) the protein content of the probiotic duck nuggets. Further test results *Duncan's* multiple range showed that the factors A and B influence factors were significantly different (P <0.05) the protein content of the probiotic duck nuggets.

Table 1 shows the factors A1 (0% of white oyster mushroom) and factor A2 (10% of white oyster mushroom) effect not significant (P> 0.05), but significant (P <0.05) at factor A3 (20% of white oyster mushroom ). This is caused by the increasing addition of oyster mushroom on a nugget cause an increase in water content and reduced meat in duck nuggets probiotics. Supported by the opinion of Utomo, Djalal and Aris (2014) study the addition of white oyster mushrooms on making nugget will cause an increase in water content.

Treatment of white oyster mushroom (factor A) closer to the levels of protein nuggets of meat intact without the addition, but it can not exceed the levels of the protein, caused by a reduction of meat into nuggets so the meat is better protein to be reduced while protein on white oyster mushrooms although the same can not be exceeded the quality of the meat protein.

At factor B1 (0% carrots), B2 (10% carrots), and B3 (20% carrots) showed a significantly different effect (P <0.05) the protein content of nuggets. Factor B (carrot) shows the tigggi Extra show decreased levels of protein. This is caused by the protein content in carrots is so low that it can not improve the levels of proteins by substituting the duck nuggets probiotics. Shown in Table 1 that the amount of protein levels increased in the treatment factor A (oyster mushroom) while treatment factor B (carrots) over 10% resulting in reduced levels of protein in the probiotic duck nuggets.

Table 1. Mean levels Nugget Ducks Probiotics Protein (%) at various Percentage Giving White Oyster Mushroom *(Pleurotus ostreatus)* (Factor A) and Carrot *(Daucus carota* L) (Factor B)

| White Oyster Mushrooms (Factor A) | Carrots (Factor B) | | | Mean |
|---|---|---|---|---|
| | B1 | B2 | B3 | |
| A1 | 16.46 | 15.58 | 15.13 | 15.72 [a] |
| A2 | 16.04 | 15.15 | 15.06 | 15.41 [a] |
| A3 | 15.65 | 15.00 | 13.05 | 14.57 [b] |
| Mean | 16.05 [a] | 15.24 [b] | 14.41 [c] | 15.23 |

Description: [abc] Mean with a smal superscript letters indicate significantly different (P <0.05)

Table 2. Mean calcium levels Nugget Ducks Probiotics (%) at Various percentage Giving White Oyster Mushroom *(Pleurotus ostreatus)* (Factor A) and Carrot *(Daucus carota* L) (Factor B)

| factor A | factor B | | | Mean |
|---|---|---|---|---|
| | B1 | B2 | B3 | |
| A1 | 0.99 [cd] | 0.97 [cd] | 0.87 [de] | 0.94 |
| A2 | 0.98 [cd] | 1:04 [c] | 1:14 [b] | 1:05 |
| A3 | 1:26 [a] | 0.94 [d] | 0.80 [de] | 1:00 |
| Mean | 1:08 | 0.98 | 0.94 | |

Description: [abcde] Mean with a small superscript letters indicate significantly different (P <0.05)

This is consistent with previous research by Melisa (2011) which states that the protein content of carrots low nugget. Best protein levels in this study is found in the treatment factor A (oyster mushroom) and factor B (carrot) at 0% with a protein content of duck nuggets of probiotics is 16:46%. The protein content is better than the levels of protein in quail sausage culled on research Son (2013) with 13.20% and equaled the protein content in Utomo research, Djalal and Aris (2014) chicken nuggets substituted with oyster mushroom is 16.50%. Nugget duck probiotics with the addition of white oyster mushrooms and carrots still meet the Indonesian National Standard (SNI) about the nugget number 6638-2014.

*Analysis of Calcium Levels Probiotics Nugget Ducks*

In Table 2 shows the average level of calcium nugget ducks probiotics with the provision of 0%, 10% and 20% of white oyster mushrooms and carrots ranged from 0.80% - 1.26% with the average levels of calcium nugget duck probiotics supreme 1.26% (126 mg / 100g) at A3B1 and the lowest was 0.80% (79.51 mg / 100g) at A3B3.

Shown in the table their interaction significantly different (P <0.05) factor A (oyster mushroom) with factor B (grated carrots) on calcium levels nugget ducks probiotics, that the treatment A3B1 with high levels of calcium 1.26% (126 mg / 100g) is the best treatment.

Results of analysis of variance showed interaction between factor A and factor B to the level of calcium nugget effect ducks probiotic significantly different (P <0.05). The results of a further test multiple range *Duncan's* show that the interaction of treatment to calcium levels nugget ducks probiotics in the treatment of A3B1 (1.26%) were significantly different (P <0.05) to the treatment A2B3 (1.14%), A2B2 (1.04%), A1B1 (0.99%), A2B1 (0.98%), A1B2 (0.97%), A3B2 (0.94%), A1B3 (0.87%) and A3B3 (0.80%). Significantly different (P>0.05) levels of calcium in duck nuggets probiotic caused by the content of calcium from oyster mushroom and carrots into substitute calcium in duck nuggets probiotics which calcium content of the oyster mushroom is higher than calcium duck meat probiotics. Interaction factor A (oyster mushroom) and factor B (carrot) shows calcium levels increased, resulting calcium substitution of factor A (oyster mushroom)

and factor B (carrots) on meat nuggets of duck probiotics. However, in line with the addition of factor B (carrot) calcium levels will decrease while the addition of factor A (oyster mushroom) increase calcium levels, this is because the white oyster mushroom vegetable material mineral sources of calcium.

In addition to organic matter and water, foodstuffs containing inorganic compounds called minerals. These inorganic compounds derived from the ash content of a food, treatment factor A (oyster mushroom) and factor B (carrots) on nugget ducks probiotics show ash content increased in A3B1, it caused the mineral content of white oyster mushrooms and carrots. In nugget ducks also tested probiotic ash where the ash content is 3.21% A3B1 an ash content highs while the lowest ash content A3B3 is 2.19%. In the treatment of A2B1 (0.98%), A2B2 (1.04%) and A2B3 (1.14%) shows the interaction of white oyster mushroom as much as 10% with increasing treatment factor B (carrot) shows the interaction of calcium levels nugget ducks probiotics, but the treatment factor A ( oyster mushroom) exceeds 10% with the addition of treatment factor B (carrot) will lower calcium levels, this indicates that the optimal to increase calcium levels by the interaction of factor A (oyster mushroom) and factor B (carrots) in the treatment of white oyster mushroom 10% and the provision of carrots up to 20%.

Interaction best increase calcium levels in this study contained on given percentage of 10% of white oyster mushrooms and carrots at 20% with calcium levels duck nuggets of probiotics is 1.14%. The calcium levels were higher than the levels of calcium in chicken nuggets with substitution young jackfruit on research Nisa (2013) is only 0.51%. Calcium levels in the probiotic ducks nugget is higher than the Indonesian National Standard (SNI) 6638-2014 of the nugget. This is due to the SNI number 6638-2014 registered nuggets are chicken nuggets with high levels of calcium levels meat 10 mg / 100g. This is in accordance with the USDA (2015) that the calcium content in chicken meat is 10 mg / 100g. However, the meat used in this study is the nugget of duck meat probiotics which after being tested calcium levels reach 81 mg / 100g higher than chicken. High levels of calcium is also caused by the concentration of calcium in the sago flour, tapioca and panir as materials for probiotic duck nuggets which calcium content of the flour is quite high.This is supported by the USDA (2015) that the calcium content of starch reached 20 mg / 100g. According Hayanto and Philip (1992) in Fadila (2012) previously stated that calcium sago reached 11 mg / 100g, and panir 10.3 mg / 100g (Ministry of Health, 1996).

*Analysis of Results The Probiotics Ducks Nugget Appearance*

*Color*

In Table 3 shows no interaction significantly different (P> 0.05) between the white oyster mushroom (factor A) and carrots (factor B) towards the organoleptic assessment nugget color duck probiotics. Seen in the table shows that the average value of probiotics organoleptic color duck nuggets ranged from 1.68 – 2.28. Organoleptic color probiotics duck nuggets highest in A3B2 treatment is 2.28, and the lowest in the treatment of A1B1 is 1.68.

Table 3. Means Rating Appearance Color Nugget Ducks Probiotics (%) at Various percentage Giving White Oyster Mushroom *(Pleurotus ostreatus)* (Factor A) and Carrot*(Daucus carota* L) (Factor B)

| White Oyster Mushrooms (Factor A) | Carrots (Factor B) | | | Mean |
|---|---|---|---|---|
| | B1 | B2 | B3 | |
| A1 | 1.68 | 2.08 | 2.24 | 2.00 |
| A2 | 2.12 | 2.08 | 2.04 | 2.08 |
| A3 | 2.12 | 2.28 | 2.16 | 2.19 |
| Mean | 1.97 | 2.15 | 2.15 | |

*Friedman's* analysis of variance results showed that the treatment factor A (oyster mushroom) and factor B (carrots) were not significantly different (P> 0.05) to the average value of color organoleptic duck nuggets produced probiotics. This shows that the of oyster mushroom and carrot no real effect on the organoleptic color on duck nuggets probiotics. Not significantly different (P> 0.05) treatment given nuggets of duck probiotic between this treatment due to treatment factor A (grated carrots) into giver natural color content of the nuggets of duck probiotics do not get the attention of the panelists for the provision of flour panir lining the nugget is the same color , so the color is visible outside the nugget color.

Yeni (2013) in his research stating the color plays an important role in the reception of food, gave pentujuk about chemical changes, the color also becomes an important attribute. Although a high value product, good taste and texture, but if the colors are less attractive, it will cause the product less attractive.Value organoletic nugget probiotic duck was better than substituting chicken nuggets with oyster mushroom on research Permadi et al. (2012) with a value of 3.48 (scale 1-5) Melisa research on carrot nuggets with a value 3.65 (scale 1-5). The results showed that the organoleptic value of color nugget probiotic treatment ducks in a row 1.68 – 2.28 in the range of love, it means the treatment is still preferred by the panelists.

*Flavor*

Table 4 shows no interactions were significantly different (P> 0.05) between factor A (oyster mushroom) and factor B (carrots) to assessment of organoleptic aromas nugget ducks probiotics, showed that the average value of organoleptic aromas nugget ducks probiotic range between 1.84 – 2.20. Organoleptic aromas probiotics duck nuggets highest in A2B2 treatment is at 2.20 and the lowest in the treatment of A1B1 is 1.84.

Table 4. Mean Rating Appearance Aroma Nugget Ducks Probiotics (%) at Various percentage Giving White Oyster Mushroom *(Pleurotus ostreatus)* (Factor A) and Carrot *(Daucus carota* L) (Factor B)

| White Oyster Mushrooms (Factor A) | Carrots (Factor B) | | | Mean |
|---|---|---|---|---|
| | B1 | B2 | B3 | |
| A1 | 1.84 | 2:16 | 2:00 | 2:00 |
| A2 | 2:04 | 2:20 | 1.76 | 2:00 |
| A3 | 1.92 | 1.84 | 1.84 | 1.87 |
| Mean | 1.93 | 2:07 | 1.87 | |

This suggests that probiotics duck nuggets that no treatment has an aroma that is typical of the aroma of duck meat though not too strong. Aroma is preferred because of the addition of plant materials such as white oyster mushrooms and carrots provide a counterweight to the aroma of meat experiencing the cooking process. Award spices will also affect aroma nuggets.

This is because the flavor has a role gives aroma, flavor, and color in the aroma of meat nugget nuggets so overcome by the probiotic duck condiments provided. In line with Maryati (2000) which states condiment used to flavor, odor, and color in cooking.

*Friedman's* analysis of variance results showed that the treatment factor A (oyster mushroom) and factor B (carrots) were not significantly different (P> 0.05) to the average value of probiotics organoleptic aroma duck nuggets produced. This shows that the administration of oyster mushroom and carrot no real effect on the organoleptic flavor to duck nuggets probiotics. Not significantly different (P> 0.05) treatment given probiotics duck nuggets between this treatment due to the distinctive aroma of the duck meat can be offset by vegetable aromas of white oyster mushrooms and carrots for processing. The results showed that the organoleptic value probiotics treatment nugget ducks in a row 1.76 - 2:20.

*Texture*

Table 5 shows no interaction significantly different (P> 0.01) in between factor A (oyster mushroom) and factor B (carrots) to assessment of organoleptic texture nugget ducks probiotics, showed that the average value of organoleptic texture nugget ducks probiotic range between 1.72 - 2:20.

Organoleptic texture probiotics duck nuggets highest in A1B2 treatment is at 2.20 and the lowest in the treatment of A2B3 is 1.72. In the treatment of carrots 10% indicate a preferred texture it is because carrots have a moisture content lower than that of white oyster mushroom formed so that the texture is more neutral. *Friedman's* analysis of variance results showed that the oyster mushroom and carrot are not significantly different (P> 0.05) to the average value of probiotics organoleptic texture duck nuggets produced. This shows that the treatment factor A (oyster mushroom) and factor B (carrots) effect did not significantly affect the organoleptic texture duck nuggets probiotics.

Not significantly different (P> 0.05) treatment given probiotics duck nuggets between treatments is due to the addition of the treatment of white oyster mushrooms and carrots which contain fiber, fiber will make being loud and uniform texture.

Table 5. Mean Texture Appearance Rating Nugget Ducks Probiotics (%) at Various percentage Giving White Oyster Mushroom ( *Pleurotus ostreatus)* (factor A) and Carrot *(Daucus carota* L) (factor B)

| White Oyster Mushrooms (Factor A) | Carrots (Factor B) | | | Mean |
|---|---|---|---|---|
| | B1 | B2 | B3 | |
| A1 | 1.80 | 2:20 | 2:00 | 2:00 |
| A2 | 1.96 | 2:20 | 1.72 | 1.96 |
| A3 | 1.92 | 2:04 | 2:00 | 1.99 |
| Mean | 1.89 | 2:15 | 1.91 | |

Good fiber content in meat duck nuggets of probiotics and the oyster mushroom and carrot are millstones affect the texture of the duck nuggets probiotics. In line with Permadi et al, (2012) which states substituting white oyster mushroom will affect the texture becomes coarse nugget.This is due to the fiber content owned oyster mushroom and milling may also affect the texture nuggets, milled meat texture is different from the texture of meat intact. This is because during the milling process is believed to occur severance of muscle fibers by a grinding machine, thus affecting the texture of ground beef. Oyster mushroom and carrot has a soft texture and easily combined with duck meat. In line with the opinion of Kurniawan (2011) in Dahlia (2014) states oyster mushroom has a texture that is similar to poultry meat is chewy, white with a relatively neutral flavor, making it easier to integrate.

Organoleptic value indicates a better result from substituting chicken nuggets with oyster mushroom on research Permadi et al (2012) with a value of 3:04 (scale 1-5) and research nugget carrots Melisa (2011) with a value of 3.60 (scale 1-5). The results showed that the organoleptic value of texture nugget probiotic treatment ducks in a row 1.72 - 2:20 in the range of love, it means the treatment is still preferred by the panelists.

*Flavor*

Table 6 shows no interaction significantly different (P <0.05) between the white oyster mushroom (factor A) and carrots (factor B) to the organoleptic assessment duck flavor nuggets probiotics, showed that the average value of probiotics organoleptic taste of duck nuggets ranged between 1.84 - 2.24. Organoleptic taste probiotics duck nuggets highest in A2B2 treatment is 2.24, and the lowest in the treatment of A2B1 and A3B1 is 1.84.

Table 6. Mean Rating Appearance Flavor Nugget Ducks Probiotics (%) at Various percentage Giving White Oyster Mushroom *(Pleurotus ostreatus)* (Factor A) and Carrot *(Daucus carota* L) (Factor B)

| White Oyster Mushrooms (Factor A) | Carrots (Factor B) | | | Mean |
|---|---|---|---|---|
| | B1 | B2 | B3 | |
| A1 | 1.88 | 2.12 | 2.00 | 2.00 |
| A2 | 1.84 | 2.24 | 1.96 | 2.01 |
| A3 | 1.84 | 2.04 | 2.20 | 2.03 |
| Mean | 1.85 | 2.13 | 2.05 | |

In the treatment of oyster mushroom 10% and 20% in the untreated carrots give small organoleptic 1.84 for oyster mushroom provides a softer texture caused by the water content although not significantly different. Treat balance showed a high value organoletik 2.24. Legowo and Nurwanto (2004) states that the water content is an important component in the food, because the water can affect the appearance, freshness, texture and taste of food. In line with that it is caused by the existence of a balancing aroma, texture, flavor from a combination of animal and vegetable materials. In materials water content will affect the effects that arise, especially the duck meat that people in general do not like is the distinctive flavor and texture. hard. A factor in treatment (oyster mushroom) and factor B (carrot) will balance it, even though the treatment was not significantly different address.

*Friedman's* analysis of variance results showed that the treatment factor A (oyster mushroom) and factor B (carrots) were not significantly different (P> 0.05) to the average value of probiotics organoleptic taste duck nuggets produced. This shows that the administration of oyster mushroom and carrot no real effect on the organoleptic taste the duck nuggets probiotics.

Not significantly different (P> 0.05) treatment given probiotics duck nuggets between this treatment due to treatment factor A (oyster mushroom) and factor B (carrot) menyimbangkan taste of duck meat probiotics so that it becomes more accepted by the panelists. The treatment process also affects the taste nuggets that will be accepted by the panelists. In fact comestible community environment that is processed through the fried process preferred. Ketaren (2008) describes this process aims to produce a product that expands and crisp, besides increasing the flavor, color and shelf life of the final product. In addition glutamic acid content in white oyster mushrooms and carrots can also cause a bad taste when cooked. Supported by Donowati (2008) which states oyster mushroom contains glutamic acid which can cause taste savory, savory and delicious.

Showed better organoleptic value of quail meat sausage with the addition of white oyster mushrooms on research Son (2013) is 1.97 (scale 1-3) and nugget carrots on research Melisa (2011) with a value of 3:10 (scale 1-5). The results showed that the organoleptic taste value probiotic treatment nugget ducks in a row 1.84 – 2.24 in the range of like, it means the treatment is still preferred by the panelists.

## Conclusion

The addition of white oyster mushroom *(Pleurotus ostreatus)* and carrots *(Daucus carota* L) at the nugget ducks probiotics shows the interaction increases significantly different calcium levels, increased calcium best is 1.14%. 15.05% protein content and organoleptic value on a scale like showing no real interaction but still in keeping with Indonesian National Standard.

## Acknowledgements

Based on the results of the study, authors suggest better provision of white oyster mushroom *(Pleurotus ostreatus)* on the product nuggets of duck meat by 10% and carrots *(Daucus carota* L) 20%.

## References

Donowati, T. 2008. Oyster Mushroom (Pleurotus ostreatus) to Improve Food Security and Environmental Rehabilitation. Jakarta.

Fadila, I. 2011. The potential of Sago in the Diversification of Food. Open University. South Tangerang.

Ketaren, S. 2008. Introduction to Technology Oils and Fats Food. Jakarta. University of Indonesia.

Maryati, H. S. 2000. Procedures for Food. Rineka Cipta, Jakarta.

Melisa, N. 2011. The Effect of Mixing Flour and Wheat Flour Tofu Dregs as a binder material Nugget Quality Of Carrot (Daucus carota L). Essay. Study Program of Agricultural Technology. Faculty of Agricultural Technology. Andalas University. Padang.

Ministry of Health, R.I. 1996. List of Food Composition. Bhratara.

National Standardization Agency. 2014. Nugget Chicken (Chicken Nugget). SNI 6683-2014. National Standardization Agency. Jakarta.

Permadi, S.N., Mulyani, S., and Hintono, A. 2012. Levels Fiber Properties Appearance dn yield Chicken Nugget substituted with White Oyster Mushroom (Pleurotus ostreatus). Journal of Food Technology Applications, Vol.1 # 4

Rokhmah, L. N. 2008. Study Levels Phytate Acid and Protein Content During Manufacture Tempe velvet bean (Mucuna pruriens) Varying Size and Length Shrinkage Fermentation. Essay. Faculty of Agriculture. Eleven University in March. Surakarta.

Trisna, W. N. 2012. Identification of Molecular and Effect of Probiotic Lactic Acid Bacteria (LAB) Origin curds from Sijunjung Against Cholesterol Levels Pitalah Ducks Meat on Genetic Resources of West Sumatra. Article. Postgraduate. Andalas University. Padang.

United States Department Of Agriculture (USDA). 2015. USDA National Nutrient Database for Standard Reference, http://ndb.nal.usda.gov/ndb/foods/show/6478?fgcd=&manu=&lfacet=&format=&count=&max=35&offset=&sort=&qlookup=TAPIOCA. (30 April 2015).

Utomo, A. H., Djalal, R., and Aris, S.W. 2014. The addition of Studies on the Quality Chemical Mushroom Chicken Nugget. Brawijaya University. Malang.

Yeni, I. U. 2013. The Effect of Smoking Lama Ayama Salted Egg With Coconut Fiber Fuels (coco fiber) Against the protein content, fat content, and Value Appearance. Essay. Faculty of Animal Husbandry. Andalas University. Padang.

# The Potential Of Dadiah From 50 Kota District, West Sumatra as a Probiotic Food Based On Total of Lactic Acid Bacteria

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## Abstract

Dadiah is a traditional food of West Sumatra. It is a spontaneous fermented by Lactic Acid Bacteria (LAB) of buffalo milk which is fermented at room temperature for 2 to 3 days in a bamboo tube. LAB is a group of bacteria that can change carbohydrates (glucose) into lactic acid. Those bacteria produce metabolic fluid, which can inhibit the growth of pathogen bacteria. The aim of this research is to know the total of LAB as a probiotic candidate of dadiah in 50 Kota District. The sampling was conducted on four farms that produce dadiah. The method of this research is completely randomized design (CRD) with four treatments, i.e. A (farm 1), B (farm 2), C (farm 3) and D (farm 4). To detect the total of LAB using plate count method with the surface spread system. The result shown that the LAB total of the overall sample was obtained between 9.2256 log CFU/gr to 9.8907 log CFU/gr.

*Keywords : buffalo milk, dadiah, lactid acid bacteria; probiotic*

## 1. Introduction

Yoghurt and Dadiah is fermented milk products which is popular to many people. The fermented products using starter *culture lactic acid bacteria* and some *milk.* Milk is the mixture of bioactive components such as vitamins, proteins, saccharides and lipids which regulate the development of gastrointestinal tract [1]. The goat milk a kimd of milk which can be processed as yoghurt, with the range of fat content from 5.33 to 6.67 % [2]. Yogurt is fermented milk product that contains the characteristic bacterial cultures such as : *Lactobacillus bulgaricus, Streptococcus thermophilus and Lactobacillus acidhopilus,so the yoghurt is resistance to the gastrict pH and has antimicrobial activities* of Lactic Acid Bacteria [3].

Dadiah is a traditional fermented buffalo milk produced especially in West Sumatra, Indonesia, which is functional as food probiotic source. It is produced by using fresh buffalo milk with fermented at room temperature for 2 to 3 days in bamboo tubes. Dadiah also contents Lactic Acid Bacteria. West sumatera is really potencial in buffalo production. The total number of buffalo in west Sumatera was noted about 117.905 heads, where 13.036 heads located in 50 Kota Regency [4][5].

Lactic acid bacteria (LAB) is a group of Gram-positive, non-spore forming, cocci or rods, which produce lactic acid as the major end product during the fermentation of carbohydrates. LAB has been associated with beneficial health effects for natural metabolite produced as achidophilin and nisin that can inhibit pathogenic bacteria, lower cholesterol, antimutagenik, improve the immune system, prevents constipation, and can produce vitamin B and bacteriocins [6]. Proteolysis activity of LAB improves availability of proteins and amino acids. LAB forms many antibacterial substances (organic acids, carbon dioxide, ethanol, hydrogen peroxide, diacetyl,

antifungal compounds, bacteriocins, antibiotics, fatty acid, phenyllactic acid) which have positive influence on shelf-life of fermented product [7][8].

The big contribution of LAB, will provide functional benefits to human body as a probiotic bacteria. Probiotics are defined by the world health organization as living organisms, when taken in sufficient amounts; provide a benefit to human health [9]. Dadiah can be categorized as a probiotic food because this product was resulted by mechanism of fermentation lactose in milk to lactic acid by activity of lactic acid bacteria [10]. The lactic acid bacteria from dadiah for each area is different. It is detection of species Lactic acid bacteria was assessed by species -specific PCR 16S RNA [11].

The aim of this research is to know the total of LAB as a probiotic candidate of dadiah in 50 Kota Regency.

## 2. Material and Methods

### *2.1 Materials*

Materials used in this research were 12 samples of dadiah from 4 Farms of buffalo farming in 50 Kota District, namely: Farm A, Farm B, Farm C and Farm D where from each Farm was taken 3 samples. The samples were brought in a bamboo tube to the Animal Husbandry Technology Laboratory of Animal Husbandry Faculty of Andalas University. Conducted tests were done to count the total of LAB contained in dadiah in 50 Kota Regency.

### *2.2 Methods*

The method of this research is completely randomized design (CRD). Datas obtained in the present research were statistically analyzed by using variance analysis (ANOVA) in completely randomized design. Duncan's Multiple Range (DMRT) was applied to separate means. Differences were considered significant at $P<0.05$ (Steel *and Torrie*., 1997)

Tabel 1. Flock Sizes, milk production of four farms selected as respondents in 50 Kota District

| | Respondents | | | |
|---|---|---|---|---|
| | Farm 1 [A] | Farm 2 [B] | Farm 3 [C] | Farm 4 [D] |
| **Address** | Jorong batu payuang , nagari batu payuang, dusun koto mandayan, lareh sago halaban, kabupaten 50 kota | Jorong batu payuang, nagari batu payuang, lareh sago halaban, kab.50kota | Jorong lareh nan panjang, batu payuang, sago halaban, kabupaten 50 kota | Jorong lareh nan panjang batu payuang, sago halaban, kabupaten 50 kota |
| **Dairy Buffalo Population/heads** | | | | |
| Total population | 4 | 4 | 2 | 2 |
| Induk | 1 | 1 | 1 | 1 |
| Dara | 2 | 1 | - | - |
| Young does | 1 | 2 | - | - |
| Young bucks | - | - | 1 | 1 |
| **Milk Production** | | | | |
| Lactation does, heads | 7 | 5 | 3 | 2 |
| Total of buffalo lactation | 1 | 1 | 1 | 1 |
| Average (L/day) | 1-1,5 | 1-1,5 | 1 | 1 |

*Total of LAB [12]*.

LAB which analysis from dadiah in 50 Kota Regency. 1 gram of Dadiah was dissolved in nine millilitres of MRS Broth and serial dilutions conducted, and then inoculated on MRS-agar (Merck) and incubated for 48 hours at 37°C under anaerobic conditions.

*CFU/gr = total of LAB x 1/serial dilutions x 1/ weight of sample*

## 3. Result and Discussion

Based on survey analysis, there are four farms in the Lareh Sago Halaban sub district which produce dadiah in 50 Kota District. Data on Farm and milk production can be seen in Table 1.

The processing of dadiah in four farms is almost the same, it is produced by using natural buffalo milk which fermented at room temperature for 2 to 3 days in bamboo tubes. The milk is neither boiled nor inoculated with any starter culture. The fresh unheated buffalo milk is placed in bamboo tubes covered with banana leaves or plastic, incubated at ambient temperature (25°C–30°C). Dadiah is a traditional food in West Sumatera, it is a fermented milk product which is storaged for 1-2 days by traditional process in a bamboo tube [13]

*Total Lactic Acid Bacteria*

This research was focused on testing of total LAB from Dadiah as a candidate of probiotic. Total LAB obtained from this research can be seen in Table 2.

Table 2. Average of total lactic acid bacteria from Dadiah in 50 Kota District

| Treatment | Total of LAB (log CFU/gr) |
|---|---|
| A | 9,8907 [a] |
| B | 9,5591[ab] |
| C | 9,2256 [b] |
| D | 9,5096 [ab] |

According to Table 2 the number of LAB ranged between 9.2256 log CFU/g - 9.8907 log CFU/g. The results of variant analysis showed that the significant effect ($P < 0.05$) of total LAB. Test of multiple range (Duncan's) showed that the number of LAB for Farm A significant ($P < 0.05$) and it's the highest compared to the other treatments, but no significant effect on treatment C. The number of LAB in the treatment B and D were significant higher than the total LAB in treatment C . Overall the difference among them is not significant. It means that the treatment gives significant effects on the number of LAB.

The total number of LAB in fermented products is influenced by two factors: they are environmental factors (temperature, pH, oxygen) and nutrients. The factors can affect microbial growth in food are: supply of nutrients, fermentation, water, pH and oxygen availability [14]. The longer time of LAB fermentation, the higher number of lactic acid. However, overall, all the treatments have the results which suit the existing standards. Based on the Codex Alimentarius [14], the number of total bacteria in accordance with the standards of probiotic drink that is more than $10^7$ (7 log CFU / g). The results of this reseach are also consistent with previous researches on total LAB, dadiah from Alahan Panjang in Solok Regency which has the LAB amount 1.46 x $10^8$ or the same as 8.1644 log CFU/g [11]. The minimum concentration of probiotic bacteria is needed to achieve therapeutic effects still controversy, but some researchers say that the therapy of doses live probiotic cells per day $10^8$ CFU/gr is a sufficient quantity to produce beneficial effects [15][16][17]. The minimum live cell of probiotic per ml product is $10^5$ CFU [18]. The result indicates that the product has met the minimum standards of the LAB.

## Conclusion

The present study concluded that, the total of Lactic Acid Bacteria from dadiah in 50 Kota District potentially to probiotic candidate. The total of LAB is ranges 9.2256 log CFU/gr to 9.8907 log CFU/gr.

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## References

[1] Donovan, S.M. 2006. Role of human milk components in gastrointestinal development: Current knowledge and future needs. J. Pediatr. 149:49-a61.

[2] Kurnia, Y.F., Ferawati, Reswati and Khalil. 2015. Prospect of Dairy Goat Production for Small-Scale enterprise in Payakumbuh West Sumatera.Pakistan Journal of Nutrition 14 (3): 141-145. ISSN 1680-5194

[3] Kurnia, Y.F., S. Yasni., B. Nurtama. 2014. Optimation Formula of Goat Milk Yoghurt and White Oyster Mushroom Powder with Mixture Design Methods. Pakistan Journal of Nutrition 13 (5): 296-302.

[4] Dinas Peternakan Provinsi Sumatera Barat, 2012

[5] Dinas Peternakan dan Kesehatan Hewan Kabupaten Lima Puluh Kota. 2015. Data Statistik Peternakan Kabupaten Lima Puluh Kota. Payakumbuh.

[6] Pato, U. 2008. Potensi Bakteri Asam Laktat yang diisolasi dari Dadih untuk Menurunkan Resiko Penyakit Kanker. J Natur Indonesia (5)2:162-166

[7] Navrhus ja, Sørhaug T (2006) Food chemistry and food processing. Blackwell Publishing Ltd, Oxford

[8] Valerio F, De bellis P, Lonigro SL, Visconti A, Lavernicocca .2008.International Journal of Food Microbiology 122: 328-332

[9] Wadher K.J *, Mahore, J.G and umekar, M.J.2010. Probiotics: living medicines in health Maintenance and disease prevention. International Journal of Pharma and Bio Sciences . ISSN 0975-6299 Vol.1/Issue-3/Jul-Sep.www.ijpbs.net Pharmaceutics

[10] Purwati. E., Arief, dan Ade,R. 2011. Teknologi Dadih ISBN 978-979-15949-8-1. Cendekia, Publishing House. Bogor

[11] Purwati.E., Husmaini, Purwanto, dan Reno PP. 2014. Molekuler Karakterisasi Bakteri Asam Laktat Isolate Dadih Air Dingin Kabupaten Solok Sumatera Barat. Vol.40 No. 2, 15 Februari : l3l-146.

[12] Purwati, E., S. Syukur, dan Z. Hidayat. 2005. *Lactobacillus sp*. Isolation of Bivicophitomega as Probiotics Lembaga Ilmu Pengetahuan Indonesia, Jakarta.

[13] Surono, I.S., Sishigaki, T., Endaryanto, A.P. 2008. Indonesia Biodiversity from Microes to Herbal aas Potential Functional Foods. J of Agriculture Shinshu University. 44(1):23-27

[14] Tampubolon, Komariah. 2008. Mikroorganisme Dalam Pangan Laut. Fakultas Perikanan dan Ilmu Kelautan. Institut Pertanian Bogor

[15] Lourens-Hatting A, Viljoen. 2001. Yoghurt as probiotic carrier food. International Dairy J. 11 : 1-17.

[16] Tannock, G.W. 1999. Probiotic: A Critical Review. Horizon Scientific Press, England.

[17] Charterist, W.P., P.M. Kelly, L. Morelli and J.K. Collins. 1998. Ingredient Selection Criteria for Probiotic Microorganism in Functional Dairy Food. Int. J. Dairy Tech. 51 (4): 123 –135.

# The effect of level flour turmeric (*Curcuma domestica* Val) ration toward carcass local duck

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## Abstract

This study aimed to determine the effect of turmeric powder in the ration of the local duck carcass. This study uses 80 species of ducks Pitalah DOD male placed on the cage box 20 units. The research method experimentally using a randomized block design (RAK) with 4 treatments and 5 weight groups as replication. The treatments were given in this study are A (without turmeric powder / control), B (given turmeric powder 0.2%), C (given turmeric powder 0.4%) and D (given turmeric powder 0.6%). The parameters observed carcass weight, the percentage of part - carcass parts (breast and thigh) and carcass percentage. The results showed administration of turmeric powder in the ration effect is not significant (P> 0.05) on carcass weight, the percentage of part - carcass parts (breast and thigh) and carcass percentage. Based on the results of this study concluded that given turmeric powder to the level of 0.6% in the diet did not affect carcass weight, the percentage of part - carcass parts (breast and thigh) and carcass percentage.

Keywords: turmeric powder, local duck, carcass

## 1. Introduction

Needs proteins derived from livestock increasing with increasing population and social welfare. To meet these needs, among others can be obtained from poultry such as chickens and ducks that contribute producing eggs and meat.

Ducks is one of waterfowl whose existence has long been fused with the life of the Indonesian people. Utilization duck as a protein source potential considering the ducks are more resistant to disease than broilers, has a pretty good adaptability and ducks have changed the efficiency of poor quality feed into meat and eggs (Akhadiarto, 2002).

Meat ducks began to demand by consumers because it has a savory taste different from other fattening poultry. This can be seen by the increase in the production of duck meat from year to year that in 2011 the production of meat reached 28 184 tonnes, followed in 2012 rose to 33 610 tonnes and in 2013 reached 36 154 tonnes (Directorate General of Livestock, 2014).

However, the duck meat has weaknesses such as low levels of meat with a high fat content when compared to the broiler. The fat content in ducks is 8.2% per 100 g, the rate is higher than the broiler is 4.8% per 100 g (Srigandono, 1997), we need a breakthrough or innovation that ducks are able to produce a good carcass quality with meat is more worthy to be accepted by consumers. Good carcass can be obtained by adding the additive materials that can spur growth. Usually additional material given antibiotics based synthetic / chemical. This material is there that is not good for humans who consume them. This is caused by the onset of effect of residues in carcasses of ducks and if the use of antibiotics is done continuously, it will lead to resistance to

antibiotics to livestock. The presence of Salmonella sp resistance to antibiotics in ducks have been reported by Istiana (1997), proved that 70 isolates of Salmonella typhimurium found to be resistant to ampicillin by (30%), neomycin (12.8%), tetracycline (11.4%), streptomycin (8.6%), trimetropin (7.1%) and chloramphenicol (5.7%). These data indicate that some preparations antibiotics have lost their effectiveness and tend to cause microbial resistance is increasingly widespread. So, to anticipate this requires the ingredients that are natural are added to feed ducks.

One of the natural ingredients that can be added to the ration is turmeric (*Curcuma domestica* Val) in the form of flour, which functioned as a feed additive for duck. Results of laboratory analysis of ruminant nutrition and food chemistry faculty of Animal Husbandry livestock Padjadjaran University (2002), that the turmeric powder containing crude protein (12.23%), crude fiber (10.85%), fat (1.67%), ash (15 , 13%), calcium (0.13%), phosphorus (1.46%) and metabolizable energy (3247.63 kcal / g), also contain essential oil (3.18%) and yellow dye / curcumin ( 9.61%) (Results of analysis of the Research Institute for Medicinal and Aromatic Plants in Sinurat et al., 2009). Kurkuminoid turmeric contains three components, namely curcumin, desmetoksikurkumin, and bisdesmetoksikurkumin (Rukmana, 1994).

According Kusumawardhani (1988) in Pratikno (2010), the provision of turmeric in the diet can increase body weight, optimize feed conversion, and lower in fat. This is supported by research Rahmat and Kusnadi (2009), which tries to give turmeric to chicken broiler age of 2 weeks with a level (0%); (0.05%); (0.1%); (0.2%) and (0.4%) in hot and cold temperatures. Turmeric is given at the level (0.2%) can increase body weight gain in hot temperatures and shows the most efficient feed conversion values compared to all levels. In hot temperatures (30-33) $^{o}$C increase in body weight of 552 g in the control became 578 g with the value of the conversion ration (1.98) were significantly lower than in controls (2.16). This is because the high heat temperatures, turmeric has active compound curcumin form hydroxyl groups that facilitate curcumin hydrogen and donates electrons to free radicals, so that free radicals to be stable (Pietta, 2000). These results are consistent with research Bintang and Nataamijaya (2006) who reported that administration of saffron (0.04%) on broiler age of 35 days combined with lempuyang as much (0.02%), substantially improve carcass weight of 1475 g (in control) into 1749 g.

Dervish, Indo and Hasiyah (1991) adds that kurkuminoid compound in turmeric, has anti-bacterial properties that can improve the digestive process by killing harmful bacteria and stimulate the wall of the gallbladder to release bile so that it can accelerate fat metabolism. Added Purwanti (2008), the mechanism of curcumin and essential oil can enhance the appetite of livestock by speeding emptying of the stomach contents. According Yuniarti (2011) turmeric can increase the working organ of digestion of poultry, because turmeric has the function of stimulating the walls of the gallbladder secrete bile and stimulates the secretion of pancreatic fluid that contains enzymes amylase, lipase and protease are useful to improve digestion of feed ingredients such as carbohydrates, fats and proteins ,

Seeing some of the functions of turmeric on top of the many positive effects, the authors are interested in raising research titled "The Effect Level Giving Flour Turmeric (*Curcuma domestica* Val) In Rations To Carcass Local Ducks".

## 2. Material and Methods

2.1 Material Research

2.1.1 Livestock duck

Animals used in this study were males aged Ducks Pitalah one day as many as 80 heads, comes from Nagari Pitalah, Tanah

Datar. In the first week is an adaptation of ducks are ducks to adjust to the new environment and the duck was introduced to the ration will be given during the study. Treatment started at the beginning of week 2 up to week 10.

2.1.2 Cages and Equipment

Cages used in this study is a wire cage crib pads sejumla 20 units with enclosure size 75cm x 60cm x 50cm. The equipment used is a feed, drinking water, incandescent 60 watt, wagon, curtains cover, black plastic is great for pedestal box, bucket, digital scales with a capacity of 5000 grams, a black plastic bag, label paper, paperboard, rice husk, knife and stationery.

2.1.3. Rations Experiment

Materials used ration consists of: yellow corn, rice bran, soybean meal, fish meal, mix top and palm oil. Denaturant additive (turmeric powder) form of Turmeric from plantations owned by farmers in Koto Tuo, land in the area around the University of Andalas. Rations trial is based on the nutritional needs of local ducks by Bintang et al., (1997). Nutrient needs of local duck meat can be seen in Table 1, the content of nutrients and metabolic energy constituents of the ration can be seen in Table 2, the composition of the materials making up the ration can be seen in Table 3, and the content of nutrients and metabolic energy of the ration treatment can be seen in Table 4.

2.2 Research Methods

2.2.1 Research Design

This research was conducted by the experimental method, using a randomized block design (RBD). Ducks are used as many as 100 birds. Adaptation ducks for 1 week, then duck totaling 100 individuals have as many as 80 species and grouped into 5 groups based on body weight ranging from the lowest to the highest. Furthermore ducks were placed into a cage with a box of 20 units each - each unit enclosure contains four ducks randomly. In one group was given 4 different treatment turmeric powder. The treatment consists of:

1. Treatment 1 was given turmeric powder in the ration experiment many ducks as (0%) per ration, called A as a control.
2. Treatment of turmeric powder 2 is supplied in the diet as many ducks trial (0.2%) per ration, which is referred to as B.
3. Treatment is given turmeric powder 3 in the diet as many ducks trial (0.4%) per ration, which is referred to as C.
4. Treatment of 4 is given turmeric powder in feed ducks trial as much (0.6%) per ration, known as D.

2.2.2 Implementation Research

The research was conducted in the form of an experiment with the stages of preparation, adaptation and treatment. In the preparation phase, the activities undertaken are made turmeric powder, set up and clean up where maintenance is used in such a way that cattle are comfortable, prepare the equipment enclosure and the procurement of feed materials research.

1. Preparation of powder turmeric (*Curcuma domestica* Val)

Turmeric powder made by turmeric washed first, pearled outer skin which is still lagging behind the roots and soil, then sliced thin - thinner.

**Table 1. Nutrients required by the Local Ducks**

| Nutrient | Required | |
|---|---|---|
| | **0-4 weeks** | **4-8 weeks** |
| Protein (%) | 20 | 16 – 18 |
| Metabolis energy (kkal/kg) | 2900 – 3000 | 2500 – 2800 |
| Crud fiber (%) | 7,60 | 8,20 |
| Ca (%) | 0,9 - 1,2 | 0,9 - 1,2 |
| Fosfor avialale (%) | 0,7 – 0,9 | 0,7 - 0,9 |

*Source : Bintang et al., 1997*

**Table 2. The content of nutrients and metabolic energy constituents of the ration :**

| Pakan | CP (%) | ME (kkal/kg) | CF (%) | CFib (%) | Ca (%) | P (%) |
|---|---|---|---|---|---|---|
| corrn* | 8,04 | 3370 | 2,66 | 4,57 | 0,37 | 0,1 |
| Rice mealss** | 10,60 | 1630 | 4,09 | 10,84 | 0,70 | 0,06 |
| Crude of o soybean* | 39,87 | 2240 | 1,67 | 0,29 | 1,3 | 0,29 |
| Flour o fissh* | 45,34 | 3080 | 3,55 | 2,89 | 3,42 | 1,31 |
| Top mix** | - | - | - | - | 5,38 | 1,14 |
| Palm oil* | - | 8600 | 100 | - | - | - |

*Source: * Wizna et al., 2008 ** Wahju (1997)*

**Table 3.** The composition of the materials making up the ration can**:**

| Nuttrition | Acount (%) | |
|---|---|---|
| | Ration 1 (starter) | Ration 2 (grower) |
| Corn | 50 | 49 |
| Rice meal | 15 | 22 |
| Crude of soyban | 14 | 13 |
| Flour ofTurmeric | 20 | 15 |
| Top Mix | 0,5 | 0,5 |
| Minyak sawit | 0,5 | 0,5 |
| Total | 100 | 100 |

**Table 4.** The content of nutrients and metabolic energy of the ration treatment

| Nutrition (%) | Ration 1 | Ration 2 |
|---|---|---|
| Protein | 20,26 | 18,26 |
| Fat | 3,39 | 3,45 |
| Crude Fibre | 5,40 | 5,91 |
| Calsium | 1,19 | 1,05 |
| Fosfor | 0,37 | 0,30 |
| Energi metabolis (Kkal/kg) | 2902,1 | 2806,1 |

*Explained: a count based of table 2 dan 3*

The slices are then aerated turmeric - aired for two days and put into oven with a temperature of ± 50 ° C for 1 day. Turmeric is then pulverized in a blender and filtered using a sieve into turmeric powder.

2. Preparation of the Cage

Two weeks before the DOD entered, the cage is cleaned beforehand using water and detergent, then spraying the enclosure using formades that has been diluted with water, then liming. One day before the DOD entered, the cage is cleaned again, be the installation of incandescent lamps for each cage box and covered with a plastic curtain.

3. Preparation ration research

The materials making up the ration research each weighted according to the composition of the ration treatment, then stir until evenly distributed. Stirring began with little material composition and continued with more material until it looks homogeneous composition.

4. Treatment and placement of ducks in cages

Placement of treatment for each - each unit is done randomly in groups, namely by writing the letters and numbers on paper in accordance with the number of treatments and the group, namely: A1-A5, B1-B5, C1-C5, D1-D5. The numbers on the paper placed on the treatment enclosure.

Table 5. Requirements ration Ducks Age 0-10 weeks

| Age (Weeks) | Ration/head/day (g) |
|---|---|
| DOD - 1 | 15 |
| 1 - 2 | 41 |
| 2 - 3 | 67 |
| 3 - 4 | 93 |
| 4 - 5 | 108 |
| 5 - 6 | 115 |
| 6 - 7 | 115 |
| 7 - 8 | 120 |
| 8 - 9 | 130 |
| 9 - 10 | 145 |

Description: Wakhid (2013)

5. Maintenance

Rationing is done in accordance with the needs of duck / day according Wakhid (2013), can be seen in Table 5. Rations are given three times a day ie morning (at 8:00 to 09:00 pm), lunch (13:00 pm) and evening (at 5:00 p.m. to 18:00 pm). Rations are given in the form of crumb (mash) ad libitum and drinking water are provided ad libitum.

6. Slaughter

Things - things to do in preparing for live ducks into carcasses are as follows:

1. gratification. Before the cut ducks were fasted for 12 hours to reduce the content of the digestive tract.
2. Slaughter of . Ducks is cut right on the neck close to the head, by cutting the jugular vein, carotid artery, esophagus and trachea.
3. Expenditure blood. After being cut ducks were left hanging with the head facing down for approximately 1 minute so that some of the blood out, then weighed.
4. Brewing (scalding). Ducks dipped in hot water at a temperature of about 90o C for ± 1 minute for easy hair removal.
5. Eviserasi. After manual plucking by hand, followed by evisceration of the abdominal cavity is done by making a horizontal incision in the abdominal area which is between the end of the sternum to pubis. Viscera pulled out by hand slowly. Furthermore, the cutting head, neck and legs. Carcasses were weighed as a whole.

6. Separation of the thigh and chest, and then weighed.

3.2.3 Variables Observed

Carcass is part of a bird's body without blood, feathers, neck, head, legs and internal organs except the lungs - lungs and kidneys (Mulyono, 2004).

Thus, several variables were observed in this study are:

a. carcass weight

Carcass weight is the weight of the body after deducting duck feathers, blood, internal organs except the lungs and kidneys, neck, head and legs (mottle and Avens, 1985). Carcass weight is calculated in grams / tail.

b. The percentage of part - carcass parts (the percentage of the thigh and chest)

The percentage of carcass parts is obtained by dividing the weight of carcass parts (breast and thigh) with a carcass weight and then multiplied by 100% (Soeparno, 2005). The weight of the chest consists of the skin which is in the chest and the meat attached to the sternum bone along his bones. As for the weight of the thigh consists of the skin that are in the thigh, the flesh attached to the pelvic bone and femur plus meat separated at the joint between the femur and tibia.

c. percentage carcass

Carcass percentage is the ratio between carcass weight with a body weight is then multiplied by 100% (Soeparno, 2005).

## 3. Result and Discussion

3.1 The Effect of the Treatment of Carcass Weight

The average carcass weight of duck with the provision of turmeric powder

(Curcuma domestica Val) during the study are shown in Table 6.

Table 6. Mean Weight Per-head Duck Carcasses For Research

| Treatment | carcases (g/head) |
|---|---|
| A (0,0% Flour ofTurmeric) | 881,80 |
| B (0,2% Flour ofTurmeric) | 869,80 |
| C (0,4% Flour ofTurmeric) | 857,00 |
| D (0,6% Flour ofTurmeric) | 863,60 |
| SE | 25,49 |

Description: Different was not significant (P> 0.05)

Results of analysis of variance shows that administration of powder turmeric (*Curcuma domestica* Val) in ration ducks showed no effect (P> 0.05) on carcass weight. This is because the provision of turmeric powder in an amount which can still be tolerated by ducks, from 0.2-0.6% in the ration has not been able to significantly influence carcass weight, so that the carcass weight gained nearly the same relative to the carcass weight control diet. In addition, administration of turmeric powder to the level of 0.6% does not affect the palatability of duck. This can be seen in the results of feed intake were not significant that treatment A (5173.85 g), treatment B (5108.65 g), treatment C (5252.73 g) and treatment D (5163.20 g). Although Purwanti (2008) suggested that curcumin contained in turmeric, has properties that can affect appetite by speeding emptying of the stomach so the appetite increase and accelerate spending bile in increasing the activity of the gastrointestinal tract. Allegedly, that the invisibility effect of turmeric powder in feed rations are also due to all treatment is based isoprotein and isoenergi, in accordance with the standard requirements in ducks , by equating the need for protein (feed phase starter as much as 0-4 weeks 20.26% and grower rations phase of 5-10 weeks of age as much as 18.26%) and energy (in the starter phase 2902.1 kcal / Kg and grower phase 2806.1 kcal / Kg). As stated Sudiyono and Purwatri (2007) that the carcass weight is affected by the consumption of feed, energy and protein content.

Rationing is also ad libitum, have an impact on feed consumption is relatively the same that gives the same effect on the body weight that treatment A (1391 g), treatment B (1378.40 g), treatment C (1349.80 g) and treatment D ( 1362.60 g) and carcass weight to be relatively equal. Anggorodi (1995) also states that the ducks were given rations ad libitum, would eat primarily to meet its energy needs and if the ducks were given rations with metabolizable energy content is low, then the ducks will consume more. This is in accordance with the opinion of Wahju (1997), that the amount of feed consumed will determine the weight of life gained, the more rations consumed also increase the live weight produced, as well as carcass weight.

According to Ricardo (2014), the average carcass weight of ducks Pitalah 8 weeks old with extensive maintenance system is 464.14 g. While the results of studies using turmeric powder to 0.6% in the average carcass weight ration obtained is higher at 857 to 880.81 g at the time of harvest age of 10 weeks. This is because the system maintenance, age and different feed.

The results using turmeric powder up to 0.6% in the average carcass weight ration obtained almost identical to the results of research Chakra, Siti, Wiyana and Umiarti (2009) ie carcass weight Bali ducks aged 10 weeks ranged from 854 to 915.40 g using polar and Duck Mix additive as a partial replacement of commercial ration. But the results of this study is lower than the Matitaputty study (2002) that gets the average carcass weight Mandalung ducks aged 10 weeks of 1101.2 g. Differences in the carcass weight were produced, due to the type of feed given ducks and different. In accordance with the opinion of Tambunan (2007), that the carcass weight is influenced by the type of ducks, quantity and quality of rations in

addition to body weight, fatty, gender, age and activity. In addition, the rate of growth of livestock, genetic and non-carcass weight also affects the resulting carcass weight (Soeparno, 2005).

3.2. Treatment effect Against Percentage Carcass Parts (Breast and Thigh)

The average of the percentage of part - part duck carcass with the provision of turmeric powder (*Curcuma domestica* Val) during the study are presented in Table 7.

Results of analysis of variance shows that administration of powder turmeric (*Curcuma domestica* Val) in ration ducks showed no effect (P> 0.05) on carcass weight. This is because the provision of turmeric powder in an amount which can still be tolerated by ducks, from 0.2-0.6% in the ration has not been able to significantly influence carcass weight, so that the carcass weight gained nearly the same relative to the carcass weight control diet. In addition, administration of turmeric powder to the level of 0.6% does not affect the palatability of duck. This can be seen in the results of feed intake were not significant that treatment A (5173.85 g), treatment B (5108.65 g), treatment C (5252.73 g) and treatment D (5163.20 g).

Rationing is also ad libitum, have an impact on feed consumption is relatively the same that gives the same effect on the body weight that treatment A (1391 g), treatment B (1378.40 g), treatment C (1349.80 g) and treatment D ( 1362.60 g) and carcass weight to be relatively equal. Anggorodi (1995) also states that the ducks were given rations ad libitum, would eat primarily to meet its energy needs and if the ducks were given rations with metabolizable energy content is low, then the ducks will consume more. This is in accordance with the opinion of Wahju (1997), that the amount of feed consumed will determine the weight of life gained, the more rations consumed also increase the live weight produced, as well as carcass weight.

According to Ricardo (2014), the average carcass weight of ducks Pitalah 8 weeks old with extensive maintenance system is 464.14 g. While the results of studies using turmeric powder to 0.6% in the average carcass weight ration obtained is higher at 857 to 880.81 g at the time of harvest age of 10 weeks. This is because the system maintenance, age and different feed.

Table 7. Percentage Mean Duck Breast and Thigh Per Head For Research

| Treatment | Breast (%) | Percentage of Thigh (%) |
|---|---|---|
| A (0,0% Flour of Turmeric) | 27,20 | 24,50 |
| B (0,2% Flour of Turmeric) | 27,99 | 24,70 |
| C (0,4% Flour of Turmeric) | 26,98 | 24,13 |
| D (0,6% Flour of Turmeric | 26,95 | 24,01 |
| SE | 0,71 | 0,49 |

Description: Different was not significant (P> 0.05)

Table 8. Percentage Mean Duck Breast and Thigh Per Head For Research

| Treatment | Breast (%) | Percentage of Thigh (%) |
|---|---|---|
| A (0,0% Flour of Turmeric) | 27,20 | 24,50 |
| B (0,2% Flour of Turmeric) | 27,99 | 24,70 |
| C (0,4% Flour of Turmeric) | 26,98 | 24,13 |
| D (0,6% Flour of Turmeric | 26,95 | 24,01 |
| SE | 0,71 | 0,49 |

Description: Different was not significant (P> 0.05)

The results using turmeric powder up to 0.6% in the average carcass weight ration obtained almost identical to the results of research Chakra, Siti, Wiyana and Umiarti (2009) ie carcass weight Bali ducks aged 10 weeks ranged from 854 to 915.40 g using polar and Duck Mix additive as a partial replacement of commercial ration. But the results of this study is lower than the Matitaputty study (2002) that gets the average carcass weight Mandalung ducks aged 10 weeks of 1101.2 g. Differences in the carcass weight were produced, due to the type of feed given ducks and different. In accordance with the opinion of Tambunan (2007), that the carcass weight is influenced by the type of ducks, quantity and quality of rations in addition to body weight, fatty, gender, age and activity.

In addition, the rate of growth of livestock, genetic and non-carcass weight also affects the resulting carcass weight (Soeparno, 2005).

3.2. Treatment effect Against Percentage Carcass Parts (Breast and Thigh)

The average of the percentage of part - part duck carcass with the provision of turmeric powder (*Curcuma domestica* Val) during the study are presented in Table 8.

Results of analysis of variance (Appendix) shows that the provision of flour turmeric *(Curcuma domestica* Val) in ration ducks showed no effect (P> 0.05) on the percentage of the chest and thigh. This is because the provision of turmeric powder up to 0.6% can still be tolerated by the ducks, so not working optimally in promoting digestion and growth in ducks including the growth of the thighs and chest. Although the Bintang and Nataamijaya (2006) reported that turmeric powder contains curcumin which is antibacterial to inhibit bacterial growth, especially in the gastrointestinal tract thereby enhancing growth, while turmeric essential oil is bacteriostatic against E.coli.

Provision of turmeric powder to 0.6% no real effect on the percentage of the thigh and chest (P> 0.05) are also due to the provision of turmeric powder no real effect on carcass weight (P> 0.05) carcass weight is obtained at treatment A (881.80 g), treatment B (869.80 g), treatment C (857 g) and treatment D (863.60 g) as a determinant of the size of the chest and thighs are relatively the same, so the percentage of part chest and thighs are relatively the same. This is because the percentage of the chest and thighs obtained from the comparison between the breast and thigh weight divided by the weight of the carcass, and then multiplied by 100% (Soeparno, 2005).

According to Lestari (2011), the percentage of the chest in ducks Alabio male age 10 weeks amounted to 31.88%. While the results of research using turmeric powder to 0.6% in the ration obtained a percentage of the lower part of the chest that ranged from 26.95 to 27.99%. However, these results are not much different from the research conducted Sudiyono and Purwatri (2007) that use enzymes in the ration of local duck male age 10 weeks, obtained a percentage of the chest of 26.96 to 29.02%. When compared with the research conducted Amaludin et al. (2013), the percentage of the chest is obtained in ducks culled Mojosari ranged from 21.87 to 23.78%, the research using turmeric powder to 0.6% in the ration is higher.

On the thigh, according to Sudiyono and Purwatri (2007) obtained a percentage of local male duck thigh at the age of 10 weeks with the addition of enzymes in rations ranged from 24.72 to 26.14%, not much different from the results of this study using turmeric powder to 0 , 6% in the ration obtained the average percentage of thigh ranged from 24.01 to 24.70% at the age of 10 weeks harvest ducks. However, these results are lower than research conducted by Amaludin et al. (2013) to salvage the Mojosari duck thigh a percentage of 34.65 to 35.13%. The

difference of the results of this study allegedly by breeds and types of feed used.

Merkley et al. (1980) states that the feed is one of the factors that influence the percentage cuts on poultry carcasses consists of the chest, back, thighs and wings. The existence of food is very important for ducks because they contain substances - nutrients needed for the formation of carcass parts and components of the body (Rasyaf, 1989). The thigh is part of its growth earlier than other sections (Swatland, 1984). According Natasasmita (1990), the thigh in ducks showed the same growth rate with the body as a whole, in other words, has a thigh isogonik growth patterns or growth that is balanced with the development of his body while breasts have heterogenik growth that naturally caused by genetic factors.

3.3. The Effect of Treatment of Percentage Carcasses

The mean percentage of carcass ducks by administering turmeric powder (*Curcuma domestica* Val) during the study are presented in Table 9.

Results of analysis of variance shows that administration of powder turmeric (*Curcuma domestica* Val) in ration ducks showed no effect (P> 0.05) on carcass percentage. This is caused by the administration of turmeric powder in an amount which can still be tolerated by ducks ranged between 0.2-0.6% in the ration, so it is not optimal to influence carcass weight and live weight as determinants of the large percentage of the carcass to be produced , Although the Stars and Nataamijaya (2006) reported that turmeric powder contains curcumin which is antibacterial to inhibit bacterial growth, especially in the gastrointestinal tract thereby enhancing growth, while turmeric essential oil is bacteriostatic against E.coli.

Table 9. The Mean Percent Per-Head Duck Carcasses For Research

| Treatment | Carcass (%) |
|---|---|
| A (0,0% Flour of Turmeric) | 63,35 |
| B (0,2% Flour of Turmeric) | 63,06 |
| C (0,4% Flour of Turmeric) | 63,47 |
| D (0,6% Flour of Turmeric) | 63,35 |
| SE | 0,38 |

Description: Different was not significant (P> 0.05)

Chattopadhyay et al. (2004) also mentions that curcumin can increase plasma levels and bicarbonate secretion of the pancreas, as well as increase the activity of lipase, amylase and pancreatic trypsin secreted.

Added Yasni et al. (1983) that curcumin and turmeric essential oil in a work capable of affecting the nerves and the pituitary gland that plays a role in secreting growth hormone.

According Brake and Havenstain (1993), carcass percentage associated with sex, age and body weight. Carcasses increase with age and body weight. Chakra (1986) states that the higher slaughter weight and carcass weight it will affect carcass percentage is higher. Soeparno (2005) added a carcass percentage is also influenced by the quality of the ration and the rate of growth. Sudiyono and Purwatri (2007) states that good growth will certainly produce a high weight and increased the percentage of carcasses optimally.

The results using turmeric powder to 0.6% in contrast to research obtained by Putri (2013) that the addition of turmeric juice into the feeding of ducks (20 ml / kg of feed) can improve carcass percentage of 54.40% (control diet) until 62.51% in hybrid ducks. The differences to this research are suspected by the shape of the processing of turmeric given and breeds. However, the research results with the use of turmeric powder up to 0.6% has the average percentage of carcass higher at 63.06 to 63.47% than the juice of turmeric them.

When compared with the results of research Chakra et al., (2009) which uses polar and Duck Mix additive as a partial replacement of commercial ration in Bali ducks, earned the average carcass percentage ranged from 57.72 to 58.73% and the research conducted Sudiyono and Purwatri (2007) which uses the addition of enzymes in the diet on a local male ducks aged 10 weeks, obtained carcass percentage ranged from 52.93 to 54.78%, the results of research using turmeric powder to 0.6% even this is also higher.

Udayana (2005) states that the low percentage of carcass weight is affected by the low cut anyway, because of the low weight cut parts - each part is wasted. Iskandar (2000) explains that the increasing age of ducks from age 5 to 10 weeks to bring improvements to the carcass weight percentage of 50-58% to 59-62%.

## Conclusion

Based on the results of this study concluded that administration of powder turmeric (*Curcuma domestica* Val) in the ration to the level of 0.6% did not affect carcass weight, the percentage of part - carcass parts (breast and thigh) and carcass percentage. Provision of turmeric powder into the ration has not been effective to improve carcass of local ducks. So that needs to be done further research by adding turmeric powder into drinking water or the provision of other form

## References


Akhadiarto, S. 2002. Kualitas fisik daging itik pada berbagai umur pemotongan. Pusat Pengkajian dan Penerapan Teknologi Budidaya Pertanian. BPPT.

Amaludin, F., Soswoyo, I. and Roesdiyanto. 2013. Bobot dan persentase bagian – bagian karkas itik Mojosari afkir berdasarkan sistem dan lokasi pemeliharaan. Jurnal Ilmiah Peternakan 1 (3): 924-932.

Anggorodi. 1995. Nutrisi Aneka Ternak Unggas. Gramedia Pustaka Utama. Jakarta.

Bintang, I. A. K. and Nataamijaya, A. G.. 2006. Karkas dan lemak subkutan broiler yang mendapat ransum dengan suplementasi tepung kunyit (*Curcuma domestica val*) dan tepung lempuyang (*Zingiber aromaticum val*). Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner, Bogor, 5 - 6 September 2006 Puslitbang Peternakan, Bogor: 623 – 628.

Brake, J. G. B. and Havenstain, S. E. 1993. Relationship of sex, age and body weight to broiler carcass yield and offal production. Poultry Sci. 72: 1137-1145.

Cakra, I. G. L. O., Siti, N. I. W., Wiyana, K. A. and Umiarti, A. T. 2009. Penggantian sebagian ransum komersial dengan polar dan aditif duck mix terhadap komposisi fisik karkas itik. Jurnal Agripet, 9, 28-34.

Chattopadhyay, I., Biswas, K., Bandyopadhyay, U., and Banerjee, R. K. 2004. Turmeric and curcumin: biological actions and medicinal applications. Current Sci., 87, 44-53.

Istiana. 1997. Resistensi *Salmonella spp* isolat itik alabio terhadap beberapa antibiotik. Jurnal Ilmu Ternak dan Veteriner, 3, 106-110.

Matitaputty, P. R. 2002. Upaya memperbaiki pertumbuhan dan efisiensi pakan mandalung melalui fortifikasi pakan dengan imbuhan pakan avilamisina [tesis]. Bogor: Program Pascasarjana, Institut Pertanian Bogor.

Merkley, J. W., Weinland, B. T., Malone, G. W., and Chaloupka, G. W. 1980. Evaluation of five commercial broiler carcass. Eviscested yield and component parts. Poultry Science, 59, 1755-1760.

Mulyono, S. 2004. Memelihara ayam buras berorientasi agribisnis. Penebar Swadaya. Jakarta.

Natasasmita, A. 1990. Tumbuh kembang pada ternak. Buletin Penelitian Universitas Djuanda, 1, 45-50.

Pietta, P. G. 2000. Flavonoids as antioxidants. Reviews. Jurnal Nat Prod 63, 1035-1042.

Pratikno, H. 2010. Pengaruh ekstrak kunyit (*Curcuma domestica* val) terhadap bobot badan ayam broiler (*Gallus Sp*). Buletin Anatomi dan Fisiologi Vol. XVIII, No. 2, Oktober 2010.

Purwanti. 2008. Kajian efektifitas pemberian kunyit, bawang putih dan mineral zink terhadap performa, kadar lemak, kolesterol dan status kesehatan broiler. Thesis: Pascasarjana. Institut Pertanian Bogor.

Putri, R. A. 2013. Pengaruh penambahan sari kunyit *(Curcuma domestica Val)* terhadap persentase karkas, persentase lemak abdominal dan kadar kolesterol daging itik hibrida. Fakultas Peternakan. Universitas Brawijaya. Malang.

Rahmat, A dan E. Kusnadi. 2009. Peranan kunyit dalam memperbaiki performan ayam broiler yang mengalami cekaman panas. Seminar Nasional

Peternakan Berkelanjutan di Universitas Padjajaran, Bandung.

Rasyaf, M. 1989. Beternak Itik. Kanisius. Yogyakarta.

Ricardo, A. Y. 2014. Gambaran bobot hidup, karkas, persentase karkas dan lemak abdomen tiga jenis itik lokal di pembibitan itik "ER" Payobasung, Payakumbuh. Skripsi: Jurusan Teknologi Produksi Ternak. Fakultas Peternakan. Universitas Andalas. Padang.

Sinurat, A.P., A.R. Setioko, A. Lasmini, dan P. Setiadi. 1993. Pengaruh tingkat dedak padi terhadap performan itik peking. Ilmu dan Peternakan, 6, 21-26.

Sinurat, A.P., T. Purwadaria., I.A.K. Bintang., P.P. Ketaren., N. Bermawie., M. Raharjo dan M. Rizal. 2009. Pemanfaatan kunyit dan temulawak sebagai imbuhan pakan untuk ayam broiler. Jurnal Ilmu Ternak dan Veteriner, 14, 90-96.

Soeparno. 2005. Ilmu dan Teknologi Daging. Gajah Mada University Press. Yogyakarta.

Srigandono, B. 1997. Ilmu Unggas Air. Gajah Mada University Press. Yogyakarta.

Steel, R.G.D dan J.H. Torrie. 1995. Prinsip dan prosedur statistik, suatu pendekatan biometrik. Terjemahan. Judul asli : Principles and Procedures of Statistics, a Biometrical Approach. Penerjemah : Bambang S. Gramedia, Jakarta.

Sudiyono, T.H dan Purwatri. 2007. Pengaruh penambahan enzim dalam ransum terhadap persentase karkas dan bagian – bagian karkas itik lokal jantan. Jurnal Pengembangan Peternakan Tropis 32 (4): 270-277.

Swatland, H. J. 1984. Structure and development of meat animal. Prenticed Hall Inc. Englewood. Cliffs. New Jersey.

Tambunan, I. R. 2007. Pengaruh pemberian tepung kertas koran pada periode Grower terhadap persentase karkas, lemak abdominal, organ dalam dan saluran pencernaan ayam broiler. Skripsi Fakultas Peternakan IPB. Bogor.

Tangendjaja, B., R. Matondang dan J. Diment. 1986 .Perbandingan itik dan ayam petelur pada penggunaan dedak dalam ransum selama fase pertumbuhan. Ilmu dan Peternakan 2(4):137-139.

Udayana, I. D. G. A. 2005. Pengaruh penggunaan lemak sapi dalam ransum sebagai pengganti sebagian energi jagung terhadap berat badan akhir dan persentase karkas itik Bali. Majalah Peternakan Universitas Udayana. Denpasar, 8, 41-44.

Wahju, J. 1997. Ilmu Nutrisi Unggas. Cetakan IV. Gadjah Mada University Press. Yogyakarta.

Wakhid, A. 2013. Beternak Itik. PT Agromedia Pustaka. Jakarta.

Wizna, Abbas H., Y. Rizal., A. Dharma dan PI. Kompiang. 2008. Improving the quality of sago pith and rumen content as poultry feed through fermentation by Bacillus amyloliquefaciens. Pakistan. J. Nut, 7, 249 – 254.

Yasni, S., K. Yoshile, and H. Oda. 1983. Dietary curcuma xanthorrhiza roxb. Increases Mitogenic Responses of Splenic Lymphocytes in Rats, and Alters Populations of The Lymphocytes in Mice. J. Nutr. Sci. Vitaminol., 39. p. 345-35.

# Effect of the Form Complete Feed With Basis Fermented Palm Oil Fronds on the Content of Moisture, Crude Lipid, and Crude Protein for Ruminants

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## Abstract

This study aimed to evaluate the effect of models of a complete feed based on palm oil fronds fermented with microorganisms derived from rumen contents. Methodology This study used a completely randomized design. The four feeds treatment with four replicates each was as follows. The models of complete feed are : (R1),Wafers complete feed (R2), biscuits complete feed (R3), pellets complete feed and (R4) conventional complete feed The experimental results show that the feed treatments significantly affected for all parameter water, fat, protein content and gross energy ($P<0.01$) . Conclusion: It was concluded that palm oil fronds could be used as forage in complete feed. The effective model complete feed in this research is biscuits complete feed.

Keywords: complete feed, palm oil frond, ruminant

## 1. Introduction

The feeding of rruminant consisting of forage, concentrates, vitamins and minerals as supplements. Forage used as feed on the farmer is grass field and a byproduct of agriculture, as well as the introduction of some grass seed grass. The national infrastructure development causes limited land to get the forage source. One of solution that is being promoted in Indonesia, the poor handling of forage is the utilization of plantation waste palm fronds and leaves as a source of forage. In 2014, the total area of palm oil plantations in Indonesia was approximately 10,956,231 ha, and in the Province of Jambi, it was approximately 688,810 ha[1]

The production and processed palm oil plantations of food result the waste . This is available of plantation waste and processing factory. The Palm oil frond and leaves a solid waste palm oil plantations that have the potential to be used as a basal feed ruminants. Palm oil frond production reaches 40-50 pieces/tree/year, and the weight of each piece is 4.5 kg. The production of palm oil fronds isestimated to be approximately 6,400 – 7,500 pieces/year/ha the plantation (Simanihuruk, 2007). Using the waste of farm and crops as feeding has a constraint because that relatively low nutritional value, and time of post-harvest treatment (Soetanto, 2001). The low nutritional value , as protein and high fiber causes the agricultural waste of limited using as animal feed (Sofyan, 1998). However, palm oil fronds are limited as feed stuff due to their high lignin content, 30.18% (Zain, 2015).

Feeding ruminant's low-quality feedstuff can result in rumen dysfunction. Several technologies are required to improve feed quality and to optimize the rumen. The ttreatment of feed materials through fermentation biotechnology approaches are

considered easier, cheaper and environmentally friendly if compare with chemical treatment. Astuti *et al.*(2012) reported that the use of local wastes, such as local microorganism sources, is beneficial due to lower costs and easier processes. Astuti *et al.* (2016) found 8 isolates of thermophilic, gram- positive bacteria among microorganisms in the rumen content. This research aims to exploit the potential of waste palm oil plantations in the leaves and palm fronds as ruminant forage with increasing their quality through innovation biotechnological fermentation of feed.

Complete feed is a feeding strategy in which roughage and concentrates in a specific proportion are mixed and formed into a specific shape. This complete feed has good nutritional value for maintenance and production. Complete feed be formed and given without any additional the other feed except water and it's capable to maintenance and production substance (Purbowati, 2009). Ginting (2009) stated that complete feed can be used to improve the quality of wet byproducts. Therefore, the present research was conducted to evaluate the nutrient value of a complete feed based on palm oil frond fermented by microorganisms derived from rumen contents.

Retrani et al (2009), the wafer is a feeding wich source natural fiber in the manufacturing process and compaction pressureheating so as to have a length and the same form. The complete feed in this research presented in various forms of complete feed, in the form of wafers, biscuits, pellets, and conventional.

## 2. Materials and Methods

Fermentation process

The rumen contents were collected from cattle in fields and placed in tubes. Sugar and coconut water were added to the tubes. The tubes were then incubated for 10 days under anaerobic conditions. The palm oil fronds were chopped into small pieces using a manual chopper and then incubated with the rumen contents for 7 days (Astuti, 2016)

Complete Feed

Complete feed wafers were composed of palm oil fronds as the forage source and concentrate. To form complete feed, the concentrate was mixed with palm oil fronds and formed into a cube using hot felt. The complete feed wafers were pressed under hot conditions. Each wafer was 5x7x2 cm. Complete feed biscuit the same as with wafer to form, and the differen is no using hot felt. Hartadi et al. (1990), pellets known as the mass of material feed wich formed by pressing and compressing through mold holes mechanically

Experimental design

The study design was completely randomized with four treatments with four replicates each. The complete feed was 40% palm oil fronds and 60% concentrat. The treatments included the following: (R1), complete feed wafers (R2), complete feed biscuits (R3), complete feed pellets, and (R4) complete feed conventional**.** The observed variables included the content of moisture, crude protein, crude lipid and gross energy of the cmplete feed basis palm oil fronds.

Statistical analysis

All data were subjected to an analysis of variance[16], and significant differences were further tested by Duncan's multiple range test.

## 3. Results and Discussion

Nutritional Contents of Complete Feeds

The nutrient contents of the complete feeds are presented in Table 1. The experimental results show that the type of complete feed significantly ($P<0.01$) affected the contents of the moisture, Crude protein, and Crude lipid. R4 had the highest than the other.

Table 1. The average nutrient contents of the complete feed based on palm oil fronds

| Variables (%) | Ration Treatments | | | |
|---|---|---|---|---|
| | R1 | R2 | R3 | R4 |
| moisture | 4.64 [b] | 4.41 [b] | 6.27 [a] | 6.41 [a] |
| Crude protein | 12.99 [a] | 13.70 [a] | 12.52 [ab] | 13.98 [a] |
| Crude lipid | 4.47[b] | 4.51 [b] | 4.59 [b] | 5.28 [a] |

*Note : (R1), Complete feed wafers (R2), complete feed biscuits (R3), complete feed pellets, and (R4) complete feed conventional*

The highest moisture 6.41% on the complete feed conventional / flour, and the lowest on complete feed wafer form. Low moisture in complete feed wafer due to wafer manufacturing process through heat felts while others without heat Felts. The moisture of the total amount of water contained in food (Winarno *et al*., 1980).

The level of moisture is important to know becausequality of the feed material is determined by the amount of moisture. The varians moisture content on this research cause of r feed ingredients in the mixing process is done manually. The water content of the ration complete all have a value below 14%. The feeding wich have moisture of less than 14% have high levels of durability and storage time longer than the higher moisture (Winarno et al., 1980). The highest crude protein content (13.98%) in this study is complete feed conventional and the lowest was 12,52%. The Duncan Multiple test showed no significant difference effect between the crude protein content of R1, R2, R2, and R4, but difference effect between R3 to R4. Suspect the no denaturation protein because the heating process.

The analyse variance showed that each treatment significantly affected the crude lipid. According to the table 1 shows the crude lipid content is highest in conventional feed (5.28) in the treatment of R4, the other treatments is reduce, that caused by the splitting of complex bonds triglycerides into simpler, example in the form of fatty acids and alcohols. Most of the fatty acids formed would evaporate so that the fat content of the rough to be down. This is in accordance with the opinion of Amrullah in Makmur (2006), the crude lipid content of the coarse feed material consisting of glycerol esters, fatty acids and vitamins are fat soluble volatile

## Conclusion

It was concluded that for ruminants palm oil fronds could be used as forage form complete feed.

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## References

Astuti, T., Y. Amir, Irdawati and U. Santoso. 2016. Nutritional improvement of palm oil fronds for ruminant feedstuffs using a local biotechnological approach. Pak. J. Nutr., 15:450–454.

BPS, 2014. Statistic perkebunan Indonesia 2013-2015 kelapa sawit. , (December 2014).

Ginting. S.P., 2009. The prospect of using complete feed in goat production: a review on its utility and physical form and animal responses. Wartazoa, 19: 64-75.

Hartadi, H. , S. Reksohadiprojo, dan A.D Tilman. 1990. Tabel Komposisi Pakan untuk Indoneaia. UGM Press, Yogyakarta.

Makmur, Indrawati. 2006. Kandungan Lemak Kasar dan BETN Silase Jerami Jagung (Zea mays L) dengan Penambahan Beberapa Level Limbah WHEY. Skripsi Sarjana, Makassar: Fakultas Peternakan Universitas Hasanuddin.

Retnani, Y. S. Basymeleh, L. Herawati. 2009. Pengaruh jenis hijauan pakan dan lama penyimpanan terhadapsifat fisik wafer. Jurnal Ilmiah Ilmu-Ilmu Peternakan November, 2009, Vol. XII, No. 4.

Purbowati, E., C.I. Sutrisno, E. Baliarti, S.P.S. Budhi dan W. Lestariana. 2007. pengaruh pakan

komplit dengan kadar protein dan energi yang berbeda pada penggemukan domba lokal jantan secara *feedlot* terhadap konversi pakan. Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner. Pusat Penelitian dan Pengembangan Peternakan, Badan Penelitian dan Pengembangan Pertanian, Departemen Pertanian, Bogor. Hal: 394-401.

Simanihuruk, K., Junjungan and A. Tarigan. 2007. Utilization of oil palm fronds as basal feed for kacang goats on growing phase). Proceeding : conference of animal science and veterinary Tekhnology, 2007

Soetanto, H. 2001. Teknologi dan Strategi Penyediaan Pakan dalam Perkembangan Industri

Peternakan. Makalah Workshop Strategi Pengembangan Industri Peternakan, Makassar 29-30 Mei 2001. Makassar. Kemahasiswaan UNHAS dan Puslitbang Bioteknologi LIPI.

Winarno, F.G., S. Fardiaz dan D. Fardiaz. 1980. Pengantar Teknologi Pangan. PT. Gramedia Pustaka. Jakarta.

Zain, M.J. Rahman, Khasrad and Erpomen., 2015. In vitro fermentation characteristics of palm oil byproducts which is supplemented with growth factor rumen microbes. Pak.J. Nutr., 14: 625–628.

# AGRICULTURE

# Soil Microbes Diversity Between Hilly and Volcanic Physiography And Their Effect To Soil Fertility

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## Abstract

Organism is one of the soil-forming factors, the influence of the organism had only associated with the amount and type of vegetation but no connecting with existing presence in soil microbiology. This study tries to connect between the number and whereabouts of isolates with the soil conditions in two different physiographic namely volkanic and physiographic physiographic hills with different land uses are rice fields, forests and gardens mix. This research was conducted in Kenagarian RaoRao, sub district Sungai Tarab, district of Tanah Datar, West Sumatra province, In physiographic volcanic with quarter andesitic parent material and pumice tuff , and the physiographic hills with granitic parent material from trias era. The results showed that the amount of the bacterial population in dry land much different from the amount of bacteria obtained in land used for rice paddies. Data from P4, on the horizon A bacterial diversity found 5 groups with the number of $6x\ 10^6$ to $16x10^7$ while in horizon B obtained two groups of bacteria with the number of $15\ x\ 10^7$, up to $27x10^7$. In the paddy soil was found three groups by the number of $5x10^7$- $8x10^7$. In physiographic granite hills on the parent material on forest land is found 3- 4 groups of bacteria by the number of $18x10^7$ to $26x10^7$ in horizon A and 3 group in horizon B with $15x10^7$ to $18x10^7$. In the paddy fields discovered four groups in horizon B with number $7x10^7$ to $17x10^7$. In the area of volcanic slopes on the horizon A land uses mixed garden found three groups of bacteria with the number of $12\ x10^7$ to $25x10^7$. On the horizon B was found three groups with an amount of $5\ x\ 10^6$ to $33\ x\ 10^6$. In the wetland encountered three groups of bacteria with $8\ x\ 10^7$ to $26\ x\ 10^7$, while in the B horizon encountered one group with a number $8x10^8$.

Keyword: physiographic system, number of microbes, soil fertility

## 1.Introduction

The organism is one of the determining factors besides soil parent material, climate, reliefs and times (Yenny, 1941). The understanding of the organism in soil science more emphasis on the land uses type and vegetations, which is evidenced by morphological observation ground. In observation of the outside factors that affect on the formation of the land, one of which is the type of land use and vegetation. The land use and vegetation affected by climate type is mainly influenced by the amount of the rainfall and temperature. Later, the types of vegetation would affect the respiration of roots and will affect the acid content of bicarbonate in solution.

Influence of macro-organism particularly evident in the case of deposition of soil on the surface of the land. This process is influenced by plant, animal and human. Also, the effect of worms and termites reverse top soil layer to bottom or vice versa. On the other hands, influence of the insect, especially mole cricket in the pasture area which reversed a Microscopic can be generalized 1 gram soil which contains millions of bacteria and thousands of kilometers long hypha fungi

as well as thousands of cell protozoa and algae. This phenomenon explained also the cycle of nitrogen and calcium are two examples of cycles of biology where there is a combination of soil and biota (Buol, 1978) layer of sand into the top layer.

The soil fertility is determined by chemical factors, namely the availability of nutrients, physical factors related to root penetration and water availability, then biological factor, especially the availability of enzyme (Brady, 1990). In the term of the biology, soil fertility marked by the R/S ratio which describe the number of the bacterial population in rhizospheres with bacteria populations in rhizoplane (Sumarno 2010). Furthermore, Liu, Dong et al (2012) suggest that the microbial properties of the rhizosphere greatly influenced by plant species that grow and inundation conditions. This is an indicator that the soil drainage conditions greatly affect the growth of bacteria. Furthermore, Manzetto A.M et al (2016) stated that the catabolic response of microbial biomass has a high correlation with land use. The ideal number of microbes in the soil will be affected by the use of agricultural chemicals and also by the treatment of land clearing. Land clearing is done by burning will affect the number and activity of microbial biomass (Fonturbei et al, 2012).

Soil moisture conditions will affect the microbiological activities. Geissler et al. (2011) stated that the availability of water significantly affects microbial activity and community composition, the activity of the protease enzyme, B-glucosidase, B-glucosaminidase, exocellulase, and phospholipid fatty acid. These four types of enzymes have a very high activity in dry conditions than in conditions of high humidity. Instead, respiration and microbial biomass will decrease in the dry condition compared to the wet condition. The activities of the enzyme would also be reduced with the decrease of pH and organic carbon. This study was disclosed by Snajdr et al. (2008) which stated that the soil profile showed that the enzyme activity decreased with increasing soil depth. This decline is in line with the decrease in pH, organic carbon, humic compounds, and microbial biomass. The changes in the enzymatic activity associated with changes in the composition of microbial communities.

According to Paul and Clark (1989), the microbial cell is absorbed by organic or inorganic soil components. This can be done because the absorption body has a charge of diverse microorganisms. As explained above, the most common microorganisms in the soil are bacteria, and most of the bacteria have negatively charged. Therefore, the interaction of bacteria with clay will also give a negative charge. With given this negative charge, the bacteria will bind metal cations that had a positive charge. When these bacteria die, the metal cations will be free and available for plants consumption.

The soil moisture conditions depend on the amount of rainfall and the pore which can hold water the land. The total pore is related to soil texture and structure and level of development of the land. In areas with the regime udic humidity, rainfall amounts exceed the amount of evaporation and soil moisture are dependent upon the percentage of clay in the soil profile.

Rao-Rao village, located in Sungai Tarab district, Tanah Datar regency are formed by physiographical volcanic and hills area. Pin the term of land uses, physiographic volcanic used to mix field, while the physiographic hills used to fields and forests. This condition is interesting to conduct research on the diversity of soil microbiology at different physiographic.

## 2. Material and Method

This research was conducted in Rao-Rao village, Sungai Tarab district, Tanah Datar regency, West Sumatra Province. The methodology used was survey method. Observations of soil and soil sampling were

conducted by drilling on the several locations include the hills with forest vegetation, and the land utilized as paddy field (*sawah*). In physiographic hilly land uses mixed farms. On the other hands, the land uses for physiographic volcanic area utilize as the mix farm.

The soil samples separated for two purposes: microbial analysis and routine analysis. The soil samples for microbiological analysis were selected from 2 layers for each horizon of the soil profile, namely A and B horizon

The analysis method use in the laboratory experiment can be explained as follow:

1. Isolation method (Yanti et al 2013) and the gram method (Lelliot and Stead, 1987) for microbial analysis.
2. For routine analysis, carried out on 2.1. Texture with pipette method (Gee, G.W and J.W Bauder 1986). 2.2 C-organic method of Walkley and Black (Nelson, D.W and L.E Sommers, 1982)

## 3. Results and Discussion

### *3.1 The condition of water management research areas.*

The humidity in the study areas is calculated based on the balance of water in the area. The calculation is based on average rainfall and water loss due to evaporation and use by plants. Average annual rainfall data for 26 years from 1978 to 2004 shows the amount of rainfall is 2291 mm with the number of rainy days as much as 175 days. The climate in the study area included in type A according to Schmidt and Fergusson, i.e. tropic rain forest areas, or included in the agricultural climate zones according to Oldelman D1, i.e. the wet months 3 consecutive months hydrological conditions in this area can be seen in Table 1.

Table 1 show that the predicted outcome value evapotranspiration is 1530 mm a year. It means that the region has a surplus of water by 415 mm over the past year. These areas include the bimodal type of rain with peaks in December (266 mm) and in March, April (245mm). Meanwhile, during June, July, August, September and October, this region experienced the water deficit which the largest one in August as much as 129 mm due to the deficit of water for the month. Then,

Table 1. Water Balance in Location

| Month | CH | HH | Etc | Accumulation | Balance | Run off | $m^3$/sec | l/sec |
|---|---|---|---|---|---|---|---|---|
| January | 225 | 16 | 120 | 425 | 305 | 105 | 0,19 | 190,80 |
| February | 165 | 11 | 120 | 365 | 245 | 45 | 0,08 | 81,77 |
| March | 245 | 17 | 120 | 445 | 325 | 125 | 0,23 | 227,14 |
| April | 245 | 15 | 120 | 445 | 325 | 125 | 0,23 | 227,14 |
| May | 223 | 14 | 120 | 423 | 303 | 103 | 0,19 | 187,16 |
| June | 125 | 9 | 150 | 325 | 175 | -25 | | 0,00 |
| July | 92 | 8 | 150 | 267 | 117 | -83 | | 0,00 |
| Augustus | 104 | 9 | 150 | 221 | 71 | -129 | | 0,00 |
| September | 168 | 12 | 120 | 239 | 119 | -81 | | 0,00 |
| October | 173 | 14 | 120 | 292 | 172 | -28 | | 0,00 |
| November | 260 | 15 | 120 | 432 | 312 | 112 | 0,20 | 203,52 |
| December | 266 | 18 | 120 | 466 | 346 | 146 | 0,27 | 265,30 |
| | 2291 | 175 | 1530 | | | 415 | | 0 |

Stasiun Salimpaung 1978-2004

CH= amount of rainfall ( mm). HH= number of rainy days

although September and October have been included into the wet months according to Schmidt and Fergusson with rainfall less than 100mm, however, for their deficit highest in August, the run-off in those months had not happened.

*3.2. Soil in the Research Areas*

The soil in this study area is divided into three types, include:

1. The land on physiographic hills with granite rock evolving towards great group Hapludult
2. The soil in the hilly region bordering pumice tuff, evolving towards dystropept
3. Land on physiographic volcanic evolving towards Hapludand.

The results of the analysis of soil texture among the sites displayed in table 2, 3, and 4.

Table 3 shown that the solum in this area seem shallow. In the mix farming soil, solum is only 38 cm deep, while on land used as paddy soil solum only 20 cm depth. The paddy field (*sawah)* shows the amount of carbon is higher than the value of carbon in the fields.

Table 4 shows that the clay fraction is higher in the A horizon either on dry land or land used as rice fields. The content of C-org on a mix of garden land is much higher than the land used as rice fields.

*3.3. Conditions of microbes in the study area*

Number isolated bacteria and fungi in the study area can be seen in the following table. Table 5 shows that the number of bacterial isolated on lands uses as rice paddies higher in horizon B. The number of isolates found as many as four isolates of bacteria, mention $7x10^7$, $9x10^7$, $15x10^7$ and 17 $x10^7$. On the slope of the land, there are more in the number of bacteria found compared with the horizon A and horizon B on forest land located. In the summit area there are the same number of bacterial isolates found in between horizon A and the horizon B.

Table 2. Results of Analysis of texture and C-org Land on physiographic Hills

| Location | Land Use | Depth | % Sand | %Silt | %Clay | % Org-C |
|---|---|---|---|---|---|---|
| Bkt Gadang | Forest | 0-25 | 14,9 | 58,4 | 26,7 | 1,825 |
| (990m) | | 25-45 | 4,41 | 28,2 | 67,4 | 1,225 |
| | | 45-60 | 4,5 | 36,2 | 59,3 | 0,675 |
| | | 60-80 | 8,9 | 60,8 | 30,3 | 0,475 |
| | | 80-100 | 22,7 | 28,5 | 48,8 | 0,425 |
| Foot Hills | Sawah | 0-25 | 6,44 | 23,96 | 69,6 | 2,025 |
| (930m) | | 25-40 | 24,3 | 43,9 | 31,8 | 0,95 |
| | | 40-60 | 19,1 | 49,7 | 31,2 | 1 |
| | | 60-80 | 26,5 | 60,3 | 13,2 | 0,9 |
| | | 80-120 | 27 | 31,7 | 41,3 | 0,75 |
| Bukit Lantik | Forest | 0-25 | 23,2 | 52,2 | 24,6 | 2,65 |
| (960m) | | 25-40 | 9,24 | 27,5 | 63,2 | 1,3 |
| | | 40-80 | 6,95 | 40,2 | 52,8 | 1 |
| | | 80-95 | 14 | 47,2 | 38,8 | 0,975 |

Table 3. Soil on physiographic hills bordering the parent rock Tuffa Pumice

| Location | Land Use | depth | %sand | %silt | %clay | %Org-C |
|---|---|---|---|---|---|---|
| Bukit Kaciek | Mix Garden | 0-18 | 37 | 17,2 | 45,8 | 1,175 |
| (953m) | | 18-38 | 28,7 | 23,2 | 48,1 | 1,05 |
| Foot Hills 950m | Sawah | 0-20 | 28,8 | 48,3 | 22,9 | 2,225 |

Table 4. Results of texture analysis and C-org on volcanic physiographic

| Location | Land Use | depth | %sand | %silt | %clay | %Org- C |
|---|---|---|---|---|---|---|
| Gadung | Mix Garden | 0-20 | 25,6 | 32,6 | 41,8 | 3,275 |
| (1100m) | | 20-40 | 14,2 | 44 | 14,8 | 2,35 |
| | | 40-80 | 20 | 77,4 | 2,6 | 1,575 |
| | | 80-120 | 21,4 | 52,6 | 26 | 1,025 |
| Andaleh | Sawah | 0-20 | 20,7 | 2,6 | 76,7 | 3,05 |
| 980m | | 20-30 | 10,7 | 71,9 | 17,4 | 1,875 |
| | | 30-70 | 10,7 | 72,8 | 16,5 | 1,325 |
| | | 70-80 | 10,7 | 41,9 | 47,4 | 1,05 |
| | | 80-100 | 23,3 | 40 | 36,7 | 1,075 |
| Marana | Sawah | 0-20 | 22,9 | 38,3 | 38,8 | 2,95 |
| 940m | | 20-40 | 24,5 | 44 | 31,5 | 1,55 |
| Mejan Panjang | Sawah | 0-20 | 26,6 | 31 | 42,4 | 3 |
| 890m | | 20-40 | 27,7 | 39 | 33,3 | 2 |

Table 5. Number of Bacteria on Physiographic Hills

| Location | Land use | depth | shape | Elevation | Edge | Diameter (cm) | Warna | Gram | Population cfu |
|---|---|---|---|---|---|---|---|---|---|
| Bkt Gadang | forest | 0-25 | filamentous | flat | Filiform | 4,5 | cream | + | 12 x $10^7$ |
| (990m) | | | Irregular | Flat | Curled | 1 | cream | + | 6 x $10^7$ |
| | | | Irregular | Flat | Curled | 1,2 | cream | + | 7 x $10^7$ |
| | | | Circular | Flat | Entire | 0,5 | cream | + | 11 x $10^7$ |
| | | 45-60 | Irregular | Flat | Undulate | 0,8 | cream | + | 16 x $10^7$ |
| | | | filamentous | Flat | Filifom | 0,8 | cream | + | 26 x $10^7$ |
| Foot hill | Sawah | 0-25 | Circular | Raised | entire | 0,3 | white | + | 12 x $10^7$ |
| (930m) | | | Rhizoid | Flat | Filiform | 1,2 | cream | + | 7 x $10^7$ |
| | | 40-60 | filamentous | Flat | Filiform | 4 | cream | + | 9 x $10^7$ |
| | | | Irregular | Flat | Curled | 0,6 | cream | + | 15 x $10^7$ |
| | | | Irregular | Flat | Curled | 0,7 | cream | + | 7 x $10^7$ |
| | | | circular | umbonate | Entire | 0,6 | cream | + | 17 x $10^7$ |
| Bkt. Lantik | forest | 0-25 | Irregular | Flat | Curled | 1 | cream | + | 26 x$10^7$ |
| (960m) | | | Rhizoid | Flat | Filiform | 1,5 | cream | + | 18 x $10^7$ |
| | | 40-80 | Filamentous | Flat | Filiform | 1,5 | cream | + | 18 x $10^7$ |
| | | | Irregular | Flat | Undulate | 1 | cream | + | 15 x$10^7$ |

Table 6. Number of bacteria on the hill Physiography bounded by pumice tuff

| Location | Land Use | depth | shape | Elevation | Edge | Diameter (cm) | Colour | Gram | Population cfu |
|---|---|---|---|---|---|---|---|---|---|
| Bkt Kaciek | Mix garden | 0-18 | Irregular | Flat | Curled | 1 | cream | + | $13 \times 10^7$ |
| (953m) | | | Irregular | Flat | Curled | 0,6 | cream | + | $10 \times 10^7$ |
| | | | Circular | Flat | Entire | 0,3 | cream | + | $16 \times 10^7$ |
| | | | Irregular | Flat | Curled | 0,5 | cream | + | $5 \times 10^7$ |
| | | | Irregular | Flat | undulate | 5,5 | cream | + | $6 \times 10^6$ |
| | | 18-38 | Filamentous | Flat | Filiform | 1,9 | cream | + | $15 \times 10^7$ |
| | | | Irregular | Flat | Undulate | 1,2 | cream | + | $27 \times 10^7$ |
| Foot Hill | Sawah | 0-20 | Irregular | Flat | Curled | 1,2 | cream | + | $5 \times 10^7$ |
| (950m) | | | Irregular | Flat | Curled | 1 | cream | + | $7 \times 10^7$ |
| | | | Irregular | Flat | Curled | 1,5 | cream | + | $8 \times 10^7$ |

Table 7. Number of Bacteria in volcanic soil physiographic

| Location | Land Use | Depth | Shape | Elevation | Edge | Diameter (cm) | Color | Gram | Population cfu |
|---|---|---|---|---|---|---|---|---|---|
| Gadung | | 0-20 | Circular | Flat | Entire | 0,5 | cream | + | $14 \times 10^7$ |
| 1100m | Mix garden | | Irregular | Flat | Curled | 1,6 | cream | + | $12 \times 10^7$ |
| | | | Irregular | Flat | Curled | 0,9 | cream | + | $25 \times 10^7$ |
| | | 40-80 | Irregular | Flat | Curled | 0,9 | cream | + | $33 \times 10^6$ |
| | | | Irregular | Flat | Curled | 0,7 | cream | + | $5 \times 10^6$ |
| | | | Irregular | Flat | Curled | 0,9 | cream | + | $9 \times 10^6$ |
| Andaleh | sawah | 0-20 | filamentous | flat | Filiform | 1,8 | cream | + | $26 \times 10^7$ |
| 980m | | | Irregular | Flat | Undulate | 0,9 | cream | + | $8 \times 10^7$ |
| | | | Circular | raised | Entire | 0,8 | cream | + | $13 \times 10^7$ |
| | | 20-30 | Rhizoid | Flat | Filiform | 1,5 | cream | + | $8 \times 10^8$ |
| Marana | sawah | 0-20 | Rhizoid | Flat | Filiform | 0,7 | cream | + | $17 \times 10^7$ |
| 940m | | | Circular | raised | Entire | 0,9 | cream | + | $16 \times 10^7$ |
| | | | Irregular | Flat | Undulate | 1 | cream | + | $13 \times 10^7$ |
| | | 20-40 | filamentous | flat | Filiform | 3 | cream | + | $13 \times 10^6$ |
| | | | Irregular | flat | Curled | 1,8 | cream | + | $9 \times 10^6$ |
| | | | Irregular | flat | Curled | 1,5 | cream | + | $5 \times 10^6$ |
| | | | Irregular | flat | Curled | 1,3 | cream | + | $7 \times 10^6$ |
| | | | circular | raised | Entire | 0,3 | yellow | + | $14 \times 10^6$ |
| Mejan Pjng | sawah | 0-20 | Irregular | Flat | Curled | 0,7 | cream | + | $7 \times 10^6$ |
| 890m | | | Irregular | Flat | Curled | 0,8 | cream | + | $13 \times 10^6$ |
| | | 20-40 | rhizoid | Flat | filiform | 0,8 | cream | + | $11 \times 10^6$ |
| | | | Irregular | Flat | Curled | 1,1 | cream | + | $10 \times 10^6$ |
| | | | Irregular | Flat | Curled | 1 | cream | + | $25 \times 10^6$ |

Table 6 show that in the A horizon, there are more bacterial population numbers found which 5 kinds of isolates with amounts ranging from $6x10^6$ to $16 \times 10^7$. This amount is twice as much on lands used as rice fields, i.e. on lands used as rice fields found 3 isolates with $5 x10^7$ amount of up to $8 \times 10^7$.

Table 7 show that there is more bacterial isolates brought on the horizon A than in horizon B for the dry land, which is 12 $x10^7$ to $25 \times 10^7$. Hence, horizon B for each isolate was $5 x10^6$, $9x10^6$, and $33x10^6$. On the lands used as the rice fields, there are 120 m lower than the location of mixed farms found 3 isolates with number $8 x10^7$, $13 \times 10^7$ and 26 $x10^7$, while in horizon B only gained 1 isolates with $8 \times 10^8$. At lower elevations, there is more number of isolates found in the

horizon B, while smaller amount found in horizon A.

Conditions fungal isolates in the study area can be seen in Table 8. It shows that the horizon A slope of the land in the forest land, discovered two fungal isolates, while in the horizon B has found one isolate on the flat land in the area of the summit on forest land. Meanwhile, there are 4 isolates found in horizon B 2 isolates. The rice fields in the horizon A is not found fungal isolates, while in horizon B 6 isolates obtained.

Table 9 shows that the soil is rich with fungal isolates populations, on the horizon A in the mixed garden soil found six fungal isolates, and on the horizon B, there are 6 isolates, so there are totally 12 isolates existing. For additional, there are 4 isolates found in the land used as rice fields.

Table 10 shows that regions in volcanic physiographic rich with the number of fungal isolates. On dry land with 1100 m elevation with the mixed garden land use in the horizon A obtained 4 isolates and in horizon B 2 isolates obtained. On lands used as rice fields at an elevation of 980 m, the number of isolates in horizon A and horizon B equals with 5 for each. At an altitude of 940 m, the number of isolates horizon A decreased to 3 isolates and this number is declining at an elevation of 890 to 2 isolates, On the horizon B, the fungal isolates fixed in number of 5.

## Discussion

The research area is developing in two physiographic and three parent materials: physiographic hills and slopes of volcanic.

Table 8. Isolates of fungi in the soil on the hills physiographic

| Location | Land Use | Depth | Population | Front color | Shape colony | Texture colony | Diameter | Back colour |
|---|---|---|---|---|---|---|---|---|
| bkt Gadang | Forest | 0-25 | 2 | A. green | A. circle | A. fine | A. 1 cm | A. brown |
| 990m | | | | B. blackish green | B. circle | B. fine | B. 0,8 cm | B. Cream |
| | | 45-60 | 1 | A. gray | Circle | fine | 0,9 cm | gray |
| | | 0-25 | | | | | | |
| 930m | sawah | 40-60 | 6 | A. green | A. circle | A. fine | A. 1,5 cm | A. brown |
| | | | | B. white | B. circle | B. downy | B. 1,7 cm | B. cream + brown |
| | | | | C. black | C. circle | C. fine | C. 0,7 cm | C. black |
| | | | | D. greenish gray | D. circle | D. fine | D. 1,9 cm | D. Cream |
| | | | | E. gren | E. circle | E. fine | E. 1,7 cm | E. white |
| | | | | F. black+ orange+ brown | F. circle | F. fine | F. 1,9 cm | F. brown |
| Lantik bkt G | Forrest | 0-25 | 4 | A. brown | A. circle | A. fine | A. 2,9 cm | A. red |
| 960m | | | | B. green+ white + orange | B. circle | B. fine | B. 1 cm | B. brown |
| | | | | C. green+ brown | C. circle | C. fine | C. – | C. green + brown |
| | | | | D. gray | D. circle | D. fine | D. 1 cm | D. black |
| | | 40-80 | 2 | A. grayish white | A. circle | A. fine | A. 1cm | A. grayish white |
| | | | | B. cream | B.circle | B. downy | B. 2 cm | B. cream |

In physiographic hilly area, the soil developed on the rock leucogranite of age Triassic (Silitonga and Kastowo). On the other hands, on the slopes of volcanic soil, it developed on rocks tuff andesitic and tuff pumice with age quarter. It indicated that the land in physiographic hills is older than the land in physiographic volcanic. It was characterized by the presence of clay in horizon B on the slopes and hilltops (table 2), while the maximum paddy fields are clay in horizon A isolates from a height of 1100 m to a height of 890 m.

In physiographic, there was no visible volcanic clay mobility found on the upper horizon, and it appeared that the value of the highest C-org obtained in a mixture of garden land. C-org value was higher than the paddy fields below.

For the results of measurement on the land capability index (Yosepa, 2016), it found that the land evolved from the physiographic volcanic rocks andesitic tuffa have the highest capability index around 63.75. It was followed by the physiographic hills that border between tuffa pumice. Whereas, the lowest value of soil developed from in granite with the index about 54.

The results of calculation of microbial and fungal isolates showed that physiographic tuffa andesitic volcanic rocks have a high number of bacterial and fungal diversity. There are more diversity and the higher amount obtained in the A horizon rather than at the horizon underneath. This microbiological diversity may have a relationship with organic matter content in the soil that developed on the volcanic physiographic.

## Conclusion

From the results of this study, it can concluded that the diversity and number of bacterial and fungal isolates were the highest in the physiographic temukana volcanic rocks of andesitic tuff when compared to the physiographic hills with rocks leuco monzonite granite.

Table 9. The number of fungal isolates on physiographic hills bounded by pumice tuff

| Location | Depth | Population | Front colour | Shape colony | Texture colony | Diameter | Back color |
|---|---|---|---|---|---|---|---|
| Bkt kaciek | 0-18 | 6 | A. brown | A. circle | A. fine | A. 1,9 cm | A. red |
| 953m | | | B. green | B. circle | B. fine | B. 1 cm | B. brown |
| | | | C. black | C. – | C. fine | C. 1 cm | C. brown + black |
| | | | D. brown | D. circle | D. fine | D. 0,7 cm | D. brown |
| | | | E. black + white | E. circle | E. fine | E. 1 cm | E. brown |
| | | | F. black | F. circle | F. fine | F. 0,4 cm | F. brown |
| | 18-38 | 6 | A.purple | A. circle | A.hairy | A.1cm | A.purple |
| | | | B.gray | B.Oval | B.fine | B.1.5cm | B.yellowish white |
| | | | C.brownish white | C.circle | C.hairy | C.2cm | C. cream |
| | | | D.white | D.circle | D.downy | D.2.5cm | D.white |
| | | | E.yellowish white | E.circle | E.fine | E.1cm | E.cream |
| | | | F. green | F.Oval | F.fine | F.1,4cm | F.green |
| Sw kk bkt | 0-20 | 4 | A. brown | A. circle | A. fine | A. 2,9 cm | A. red |
| Kaciek | | | B. green+white+ Orange | B. circle | B. fine | B. 1 cm | B. brown |
| 950m | | | C. green+ brown | C. circle | C. fine | C. – | C. black + brown |
| | | | D. gray | D. circle | D. fine | D. 1 cm | D. black |

Table 10. Number of fungal isolates on volcanic physiographic

| Location | Depth | Population | Colour | Colony shape | Colony texture | Diameter | Colour |
|---|---|---|---|---|---|---|---|
| Gadung | 0-20 | 4 | A. brown | A. circle | *A. fine* | A. 2 cm | A. red |
| 1100m | | | B. green | B. circle | B. fine | B. 2 cm | B. brown + Cream |
| | | | C. white brown | C. circle | C. fine | C. 0,9 cm | C. brown |
| | | | D. dark green | D. circle | D. fine | D. 0,9 cm | D. brown |
| | 40-80 | 2 | A. dark green | A. circle | A. fine | A. 1 cm | A. brown cream |
| | | | B. dark green | B. circle | B. fine | B. 1 cm | B. Reddish brown |
| Andaleh | 0-20 | 5 | A. black | A. circle | A. fine | A. 0,9 cm | A. dark brown |
| 980m | | | B. dark green | B. circle | B. fine | B. 1,1 cm | B. brown |
| | | | C. brown | C. circle | C. downy | C. 0,9 cm | C. brown |
| | | | D. dark brown | D. circle | D. fine | D. 1,1 cm | D. brown |
| | | | E. white | E. circle | E. downy | E. 1,4 cm | E. creamy brown |
| | 20-30 | 5 | A. black | A. circle | A. fine | A. 0,9 cm | A. dark brown |
| | | | B. dark green | B. circle | B. fine | B. 1,1 cm | B. brown |
| | | | C. brown | C. circle | C. downy | C. 0,9 cm | C. brown |
| | | | D. browny black | D. circle | D. fine | D. 1,1 cm | D. brown |
| | | | E. white | E. circle | E. downy | E. 1,4 cm | E. creamy brown |
| Marana | 0-20 | 3 | A.cream | A. circle | A. hairy | A. 2,8 cm | A. cream |
| 940m | | | B. brown | B. circle | B. downy | B. 2 cm | B. gray |
| | | | C.cream | C. circle | C. fine | C. 0,5 cm | C. yellowish white |
| | 20-40 | 5 | A. dark green | A. circle | A. fine | A. 1,2 cm | A. brown |
| | | | B. white | B. circle | B. downy | B. 2,5 cm | B. white |
| | | | C. black green | C. circle | C. fine | C. 1,1 cm | C. brown + Cream |
| | | | D. white orange | D. Lonjong | D. downy | D. 1,5 cm | D.black orange |
| | | | E. creamy brown | E. oval | E. fine | E. 1,5 cm | E. brown |
| Mejan | 0-20 | 2 | A. green | A. circle | A. fine | A. 1,7 cm | A. brown |
| Panjang | | | B. green | B. circle | B. fine | B. 0,9 cm | B. brown |
| 890m | 20-40 | 5 | A. brown | A. circle | A. fine | A. 3 cm | A. red |
| | | | B. dark brown | B. circle | B. fine | B. 1,9 cm | B. creamy brown |
| | | | C. dark green | C. circle | C. fine | C. 0,9 cm | C. creamy brown |
| | | | D. dark green | D. circle | D. fine | D. 1,3 cm | D. creamy brown |
| | | | E. green | E. circle | E. fine | E. 1,5 cm | E. Cream |

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## Reference


Buol,S.W, F.D Hole, dan R.J Mc Cracken 1978. Soil Genesis and Classification, Oxford and IBH Publisihin co, New Delhi

Buckman and Brady, 1990. The Nature and Properties of Soil

Fonturbei, A Barrero, J.A Vega, A. Martin, E.Jimenes, T.Carbalos, C.Fernandez. M.Diaz, Ravina, 2012. Effect of an experimental fire and post fire stabilization treatment on Soil Microbial communities, Geoderma vol 19.51-60

Gee,G.W and J.W Bauder 1986. Particle size analysis, dalam Arnold Klute Method of Soil Analysis part 1 Physical and mineralogical method ASA, SSCA, Madison Wisconsin

Geisseler,W.R, Hormath K.M. Scrow, 2011. Soil moisture and Plant Residu Addition Interact in Their Effect on Extracellular Enzyme Activity, Pedobiologia no 54. Hal 71-78

Indrayoga,PM., I Made Sudarma., dan Ni Made Puspawati. 2013. Identifikasi Jenis dan Populasi Jamur Tanah pada Habitat Tanaman Kubis (Brassica oleracea L.) Sehat dan Sakit Akar Gada pada Sentra Produksi Kubis di Kecamatan Baturiti Tabanan. E-Jurnal Agroekoteknologi Tropika 2(3) : 184-194

Liu,Dong. Shengzuo Fang∗, Ye Tian, Xinjian Dun,2012. Variation in rhizosphere soil microbial index of tree species on seasonal flooding land: An in situ rhizobox approach, Applied SoilEcology 59(2012)1-11.

Lelliot, Stead, 1987. Methods for the Diagnosis of Bacterial Diases of plants. Oxford: Blackwell Sci. Publ.

*Mazzetto, Andre Mancebo a,∗, Brigitte Josefine Feigl a, Carlos Eduardo Pellegrino Cerria, Carlos Clemente Cerrib, 2016.* Comparing how land use change impacts soil microbial catabolic respiration in Southwestern Amazon, Brazilian Jurnal of soil Microbiology, 47(2016) 63-72

Nelson, D.W and L.E Sommers, 1982. Total organic carbon and organic matter, Page ,A.L Methods of Soil Analysis part 2 Chemical and Microbiological Properties ASA, SSCA, Madison Wisconsin

Snajdr,J, V Valaskova,V, V Merhaulova,J.Herikovait, Cajthmal, P>Baldrian 2008. Spatial Variability of Enzyme activities and microbial biomass in the upper layer of quercus Petraea forest soil, soil biology and biochemistry 40. 2068-2075

Yanti, Y., Habazar T., Resti Z., Suhalita D., 2013. Penapisan Isolat Rizobakteri Dari Perakaran Tanaman Kedelai Yang Sehat Untuk Pengendalian Penyakit Pustul Bakteri (*Xanthomonas axonopodis* pv. *glycines*). Jurnal HPT Tropika 13(1):24-34

# Application of Green Manure and Rabbits Urine Affect Morphological Characters of Sweet Corn Plant (*Zea mays* saccharata Sturt) in Lowland of Deli Serdang District

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## Abstract

This study aims to determine the effect of green manure and rabbit urine on morphological characters of sweet corn plant. This study was conducted from April 2015 through the month of June 2015 at Jl. Lubuk Pakam Batang Kuis, Aras Kubu village, Deli Serdang, North Sumatra. The location of this research with a height of 15 meters above sea level. The design used was a factorial randomized block design, which consists of two factors: The first factor is the provision of green manure with 3 levels namely $H_0$ = without giving, $H_1$ = 1.2 kg / plot, $H_2$ = 2.4 kg / plot. The second factor is the rabbit urine with 4 levels namely $K_0$ = without giving, $K_1$ = 20 ml / liter of water, $K_2$ = 40 ml / liter of water and $K_3$ = 60 ml / liter of water. The parameters observed were plant height (cm), leaf area, weight of corn cobs per plant,weight of corn cobs per plot. The data were analyzed using ANOVA and continued with different test Mean according to Duncan (DMRT) with a level of 5%. The results showed that administration of green manure significantly affected plant height, leaf area ($cm^2$), weight of corn cobs per plant (kg) andweight of corn cobs per plot (kg). Whereas urine of rabbits significant effect on plant heightand leaf area. Interaction between green manure and urine of rabbit not significant affected for all parameters. Mean of different test results according to Duncan (DMRT) note that treatment is best green manure fertilizer $H_2$ is green with a dose of 2.4 kg / plot. While on treatment of rabbit urine concentration is $K_3$ is giving with a concentration of 60 ml / liter of water.

Keywords: Sweet corn, green manure, urine of rabbits, morphological characters

## 1. Introduction

Corn as a food crop in Indonesia ranks second after rice. While based on the sequence of basic foodstuffs in the world, maize ranks third after wheat and rice. Corn production in Indonesia is still dominated on the island of Java which is about 65% and the outer islands of Java only about 35%[8],[12].

As a source of carbohydrates corn has many benefits, such as food, feed and industrial raw materials. The use of corn as food and feed materials continued to increase, while the supply is still limited. It is necessary to attempt to increase production through expansion of cropland and increased productivity. The corn crop will not provide maximum results when necessary nutrients are not sufficiently available. Fertilizing can increase yield quantitatively and qualitatively. Fertilizer is the key of the fertility of the soil because it contains one or more elements to replace the depleted elements absorbed by plants. There are two types of fertilizer often used by farmers, namely inorganic and organik fertilizers [5],[8].

The Green manure functioning as a source and a buffer of nutrients through the decomposition process and its role against the provision of soil organic matter and soil microorganisms. These organic materials

have an important role in increasing the efficiency of fertilizer use[1],[7].

Lamtoro plants used as green manure contains nutrients that plants need. Lamtoro leaves contain nutrients Nitrogen 2.0 to 4.3%, phosphorus 0.2 to 4.3% and 1.3 to 4.0% kalium[11]. In addition there are several types of urine that can be used as liquid organic fertilizerlike urine coming from the horse, buffalo, sheep, pigs and rabbit. Urine derived from rabbit has a higher nitrogen content compared with the urine coming from other animals. The content of rabbit urine fertilizer is 2.72% nitrogen, 8.7% phosphorus, 2.3% potassium, 3.6% sulfur, 1.26% calcium and 4.0% magnesium[4],[10].

## 2. Material and Methods

The study was conducted in Lubukpakam Batang Kuis, Aras Kabu village, Deli Serdang district with altitude ± 15 metres above sea level. The study was conducted in April to June 2015.Materials used in this study is the sweet corn seed varieties of sugar 75.The size of the plot is 110 cm x 110 cm, leaves lamtoro with the three levels namely $H_0$ = Without treatment, $H_1$ = 1.2 kg/plot, $H_2$ = 2.4 kg/plot. Rabbit urine with four levels namely $K_0$ = Without treatment, $K_1$ = 20 ml/liter of water/plants, $K_2$ = 40 ml/liter of water/plant, $K_3$ = 60 ml/liter of water/plant. Parameters measured were plant height, leaf area, weight of cobs per plant and weight of cobs per plot. The data were analyzed using ANOVA and continued with Duncan's Multiple Range Test (DMRT) with a level of 5%.

## 3. Result and Discussion

*3.1. Plant height*

Plant height of sweet corn the highest atgiving of green manure $H_2$that is 187.29 cm is significantly different with $H_0$ and $H_1$. While giving of rabbit urine $K_3$that is 183.71 cm significantly different from$K_0$, $K_1$ dan $K_2$.According to[11]lamtoro leaves contain nutrients Nitrogen 2.0 to 4.3%, phosphorus 0.2 to 4.3%, and potassium 1.3 to 4.0%, so the more given the green manure then nutrients that can be utilized crops as well more. Furthermore [13] stating that rabbit urine has a content of macro and micro nutrients that is good for plant.

Figure 1 shows that the plant height of corn plant with giving of green manure form positive linear relationship with the equation y = 171.5 + 0,787x and the value of correlation = 1.So also with giving of rabbit urine form a positive linear relationship with the equation y = 176.1 + 0,109x and the value of correlation = 0.867.

Plant height of sweet corn relations with giving of green manure and rabbit urine in Figure 1.

*3.2.leaf area*

Leaf area of sweet corn plant highest at giving of green manure $H_2$ namely 501.17 cm is significantly different with $H_0$ and $H_1$. While giving of rabbit urine $K_3$ namely 494.44 cm significantly different with $K_0$, $K_1$ and $K_2$.

According to [1] green manure serves as a source and a buffer of nutrients through the decomposition process and its role in the provision of soil organic matter and soil microorganisms. rabbit urine has a content of macro and micro nutrients that is good for plant.Rabbit urine contains N, P, K were higher compared with the urine of other animals, namely 2.72%, 1.1% and 0.5% [10],[13].

Table 1. Average Plant Height of Sweet Corn

| Treatment | Plant Height (cm) |
|---|---|
| **$H_0$** | 171.55c |
| **$H_1$** | 179.34b |
| **$H_2$** | 187.29a |
| **$K_0$** | 176.82d |
| **$K_1$** | 177.94c |
| **$K_2$** | 179.10b |
| **$K_3$** | 183.71a |

Testing performed by Duncan's Multiple Range Test at the 5% level

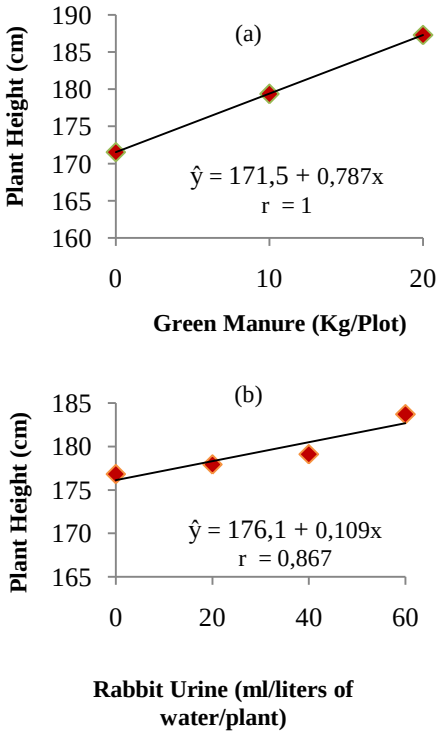


Figure 1. The Relation Plant height of sweet corn with Giving (a) green manure and (b) Rabbit Urine

Figure 2 shows that the leaf area of corn plant with giving of green manure form positive linear relationship with the equation ŷ = 433,7 + 1,575x and the value of correlation = 0,943.So also with giving of rabbit urine form a positive linear relationship with the equation ŷ = 433,9 + 1,043x and the value of correlation = 0,992.

*3.3. Weight of Corn Cobs per Plant*

Weight of corn cobs per planthighest at giving of green manure$H_2$namely0,73 kgis significantly different with $H_0$dan $H_1$. Reference [2],[9] reported that improve the physical characteristics and stimulate microbial activity of the soils. After decomposition, the organic P and K bound in the green manure crop may provide an easily accessible form of P and K to succeeding crops.

Leaf area of sweet corn relations with giving of green manure and rabbit urine in Figure 2.

Table 2. Average Leaf Area of Sweet Corn

| Treatment | Leaf Area (cm) |
|---|---|
| **$H_0$** | 438.17c |
| **$H_1$** | 456.28b |
| **$H_2$** | 501.17a |
| **$K_0$** | 432,97d |
| **$K_1$** | 454,56c |
| **$K_2$** | 478,85b |
| **$K_3$** | 494,44a |

Testing performed by Duncan's Multiple Range Test at the 5% level

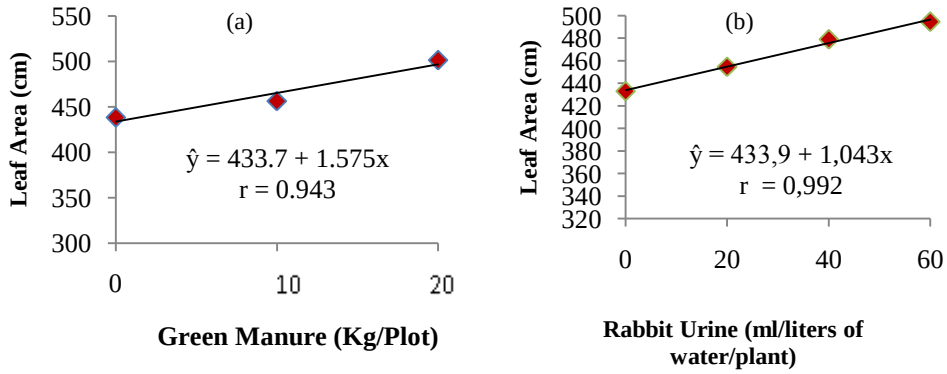


Figure 2. The Relation Leaf Area of sweet corn with Giving (a) green manure and (b) Rabbit Urine

Table 3. Average Weight of Corn Cobs per Plant

| Treatment | Weight of Corn Cobs per Plant (Kg) |
|---|---|
| **$H_0$** | 0,62b |
| **$H_1$** | 0,66b |
| **$H_2$** | 0,73a |

Testing performed by Duncan's Multiple Range Test at the 5% level

Weight of corn cobs per plantrelations with giving of green manure in Figure 3.

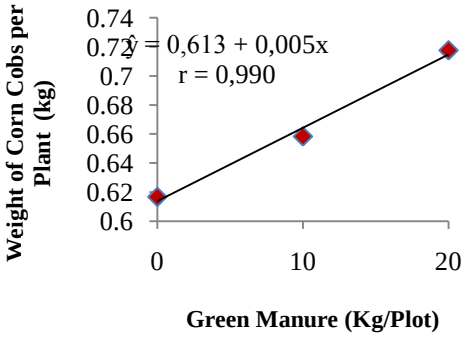


Figure 3. The Relation Weight of Corn Cobs per Plant with Giving green manure

Table 4. Average Weight of Corn Cobs per Plot

| Treatment | Weight of Corn Cobs per Plot (Kg) |
|---|---|
| **$H_0$** | 1,86b |
| **$H_1$** | 1,99b |
| **$H_2$** | 2,19a |

Testing performed by Duncan's Multiple Range Test at the 5% level

Weight of corn cobs per plotrelations with giving of green manure in Figure 4.

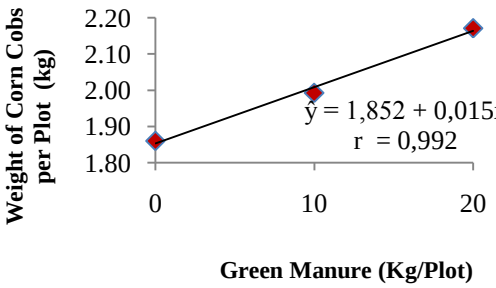


Figure 4. The Relation Weight of Corn Cobs per Plot with Giving green manure

Figure 3 shows that the weight of corn cobs per plantwith giving of green manure form positive linear relationship with the equation ŷ = 0,613 + 0,005x and the value of correlation = 0,990.

*3.4.Weight of Corn Cobs per Plot*

Weight of corn cobs per plothighest atgiving of green manure$H_2$ namely 2,19 kg is significantly different with $H_0$dan $H_1$. According to [3] reported that with green manure also, large amounts of N are applied into soil, but nutrients are released from green manure at a slower rate; also, N from N-fixing bacteria becomes accessible over a long time span. These processes grant steady sources of N for succeeding crops.

Figure 4 shows that the weight of corn cobs per plotwith giving of green manure form positive linear relationship with the equation ŷ = 1,852 + 0,015x and the value of correlation = 0,992.

## Conclusion

Giving of green manure effect on plant height, leaf area, weight of cobs per plant and weight of cobs per plot. Dosage best with the giving of green manure $H_2$ 2.4 kg / plot.Giving of rabbit urine fertilizer effect on plant height and leaf area. Dosage best with giving of rabbit urine is $K_3$ = 60 ml / liter of water.

## References

[1] A. Rosenfeld and F. Rayns,*Sort Out Your Soil: A practical guide to Green Manures*, Costwold Seeds and Garden Organic, United Kingdom. 1974.

[2] B. Eichler-Löbermann, R. Gaj and E. Schnug, "Improvement of soil phosphorus availability by green fertilization with catch crops,"

*Communications in Soil Science and Plant Analysis*, 40: 70–81, 2009.

[3] B. Freyer, *Crop Rotation*, Eugen Ulmer GmbH & Co, Stuttgart, German,2003.

[4] G. Suprijadji,"Kandungan Hara Pada Urine Kelinci, Sapi, Kambing dan Domba," Warta BPP Jember. Jember.1992.

[5] H. Budiman,*Sukses Bertanam Jagung*, Pustaka Baru Press, Yogyakarta, 2012.

[6] H. H. Glendon, Jr, *Fertilization in A Guide to Corn Production*, University of Georgia, Georgia, 2015.

[7] Juarsah, "Manfaat dan Alternatif Penggunaan Pupuk Organik pada Lahan Kering Melalui Pertanaman Leguminosa," dalam Prosiding Pusat Penelitian Tanah dan Agroklimat, Bogor, 1999.

[8] M.A. Arun Kumar, S. K. Gali and N. S. Hebsur,"Effect of Different Levels of NPK on Growth and Yield Parameters of Sweet Corn,"*Karnataka J. Agric. Sci*.,20 (1) p 41 – 43. 2007.

[9] M. Askegaard and J. Eriksen, "Residual effect and leaching of N and K in cropping systems with clover and ryegrass catch crops on a coarse sand," *Agriculture, Ecosystems and Environment*, 123: 99–108, 2008.

[10] M. Maina,"Rabbit Urine Therapy Cuts Coffee Farm Costs," Smart Farmer, Kenya,2014.

[11] N. Palimbungan, R. Labatar dan F. Hamzah, " Pengaruh Ekstrak Daun Lamtoro sebagai Pupuk Organik Cair terhadap Pertumbuhan dan Produksi Tanaman Sawi,"*J. Agrisistem* 2 (2) : 96 -101, 2006.

[12] Purwono dan R. Hartono,*Bertanam Jagung Unggul*, Penebar Swadaya, Jakarta, 2005.

[13] The Organic Farmer, "Rabbits are good for meat and income," The magazine for sustainable agriculture, Kenya, 2007.

# Humic Substance Characterization of Lignite as a Source of Organic Material

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## Abstract

Organic materials have an important role in determining the level of soil fertility, paticularly on marginal soils. Organic material which have been commonly used as compost requares a process that is long enough to be able to react in the soil. Therefore, it would require a rapid reaction of organik material such as humic substance. Humic substance can be extracted from lignite. Humic substance estimated from lignite are needed in smaller amounts than compost and other organic material sources, so as to facilitate the application and production costs. This experiment was conducted to extract lignitefor abstaining hurnic substances by using eigth kinds of extract solutions.The best and ef-fectivesolution wasinvestigated and also the characteristics of hurnic substances soluble in those solution. Complete randomize design was used to find out the effect of treatments. the ten kinds of extract solutions were 0.1 N NaOH, 0.5 N NaOH, 1 N NaOH, 0.1 M $Na_2CO_3$, 0.5 M $Na_2CO_3$, 0.1 N HCl, 0.1 M Formic Acid, 0.1 M Oxalic Acid, Ethanol 70%, and Ethanol 90%. Results of this laboratory experiment showed that all ten extraction solutions had the ability to extract humic substances but in different amount. The best solution that could be used effectively was 1 N NaOH. This solution could extract 46.5% of humic substances from lignite.

Keywords: Humic substance, lignite, soil fertility

## 1. Introduction

Organic materials have a very important role in improving soil fertility, particularly on marginal soils widespread in Indonesia. Commonly used organic materials such as manure, green manure and compost are needed in large numbers and requires a fairly long proccess to be able to react in the soil. Therefore needed faster reaction of organic materials and the most active in the soil with greater electrical charge such as humic materials that can be extracted from lignite.

Humic substance contained in an assortment of lignite and coal referred to as humic materials geology, because the old process largely fulfat acid pressure and polymerized into humic acid trough the process of diagenesis [1].

Coal reserves are many and widespread. Estimated there are more than 984 billion tonnes of coal reserves are spread throughout the world more than 70 countries. Coal foun in Indonesia reached about 38,8 billion tons, 70 % are young coal and the remaining 30 % is an old coal with high quality. This potensial should be realized by all section of society so that the management of coal optimally in the interest of the nation can continue to be considered[2.]

Lignite which can not be used as a fuel and have a deposit that much in Indonesia, so it can be used as a sorce of humic materials that can be extracted in large quantities. Thus the use of lignite to be more

effective.This research aims to explore the potensial of lignite as a source of organik material that can be used in agricultural activities and to determine the dissolved humic material characteristics of coal.

## 2. Material and Methods

This research was carried out from the month of august to October 2016 in soil laboratories Dharmasraya and pharmaceutical laboratories Andalas University. Coal used in this research was taken from the lignite Pasaman of west Sumatra-Indonesia.

Solvent used in the extraction is 0.1 N NaOH, 0.5 N NaOH, 1 N NaOH, 0.1 M $Na_2CO_3$, 0.5 M $Na_2CO_3$, 0.1 N HCl, 0.1 M Formic Acid, 0.1 M Oxalic Acid, Ethanol 70%, and Ethanol 90%. Design used in this research is completely randomized design with 10 treatments and 4 replications. The observed data were statistically analyzed using the fisher test and if significantly different will be followed by Duncan New Multiple Range Test (DMNRT) 1 %.

### *2.1. Method of extracting humic substance from lignite*

Weigh 10 g of lignite, was added to 50 ml centrifuge tube that has been weight. Enter 10 ml of solvent, shaken 30 minutes. Centrifuge at a speed of 4000 rpm for 15 minutes, pour aliquots into 100 ml flask through a funnel that has been given a filter paper. Centrifuge tube dry in an oven at a temperature of 80 $^oC$ and eventually weigh heavily.

## 3. Result andDiscussion

The result of this research indicate that each solvent have different capacities to extract the humic substance from lignite.10 kinds of solvents, it is knows that NaOH 1 N a solvent that is most effective in extracting humic substance from lignite that is able to extract 46.8 % of humic substance.

Table 1. the ability of 10 types of solvent in extracting humic materials from lignite

| No | solvent | Humic substance (%) |
|---|---|---|
| 1 | 1 N NaOH | 46.8 |
| 2 | 0.5 N NaOH | 38.4 |
| 3 | 0.1 N NaOH | 26.5 |
| 4 | 0.1 N $Na_2CO_3$ | 14.2 |
| 5 | 0.5 N $Na_2CO_3$ | 22.9 |
| 6 | 0.1 N HCl | 7.9 |
| 7 | 0.1 M Formic Acid | 6.7 |
| 8 | 0.1 M Oxalic Acid | 9.4 |
| 9 | Ethanol 70 % | 5.1 |
| 10 | Ethanol 90 % | 6.3 |

The ability of each solvent can be seen in Table 1. The result of the analysis of functional groups of humic substance from lignite using infra red analysis can be seen in figure 1.

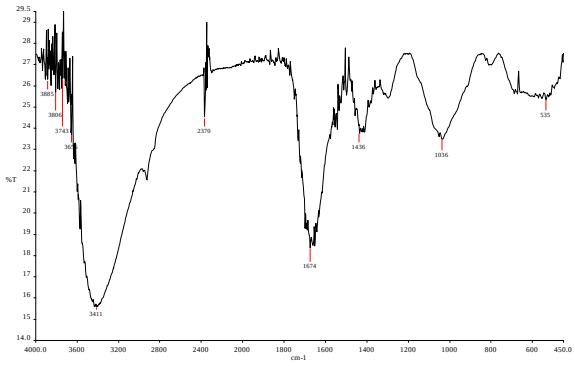


Figure1. ……

The analysis of functional groups of humic substance from lignite using infra red analysis can be stated that the humic substance has a functional group and a series of O-H and N-H circuit at wave number 3367-3476 $cm^{-1}$. It is the hallmark of which is owned humic substance.

At 3300 – 3400 wave numbers for the uptake of humic substance that is a series of

O-H and N-H circuit. Humic acid not expected to have a group of C-H that usually appears at wave number 730 – 900 $cm^{-1}$[3].

## Conclusion

10 kinds of solvents, it is knows that NaOH 1 N a solvent that is most effective in extracting humic substance from lignite that is able to extract 46.8 % of humic substance. The analysis of functional groups of humic substance from lignite using infra red analysis can be stated that the humic substance has a functional group and a series of O-H and N-H circuit.

## Acknowledgements

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## References

[1] Tan, K.H. Humic matter in soil and environment. Principles and Controversies. University of Georgia. Athens, Georgia. USA 386 p. 2003

[2] Raharjo. Mengenal batubara. Artikel iptek bidang energi dan sumber daya alam. Berita iptek.8 p. 2006

[3] Orlov. DS. Humus acid of soils. Amerind.Publ. Co. New Delhi. 378 p. 1985.

# Nutritional Composition of Ruminant Forage Derived from Rice Crops (*Oryza Sativa* L.) that Applicated by *C. odorata* Compost

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## Abstract

The study about Nutritional Composition Of Ruminant Forage Derived From Rice Crops (*Oryza Sativa* L.) that Applicated By *C.odorata* Compost had been conducted in the District Koto Tangah, Padang, West Sumatra began in June 2015 through October 2015. The study aimed to get good quality forage and yield in the cultivation of two varieties rice crops that applicated *C.odorata* compost. Varieties tested were Pandan Wangi and Cisokan. The experiment was arranged in the split plot design. The main plot there were 2 levels cutting rice forage, which was not cut (Po) and cut as high as 15 cm of the soil surface (P1). The subplot was *C.odorata* compost (CC) combined with recommendation fertilizer dose (RFD) consisting of 3 levels; B1. 5 Mg $ha^{-1}$ CC + 100% RFD; B2. 7,5 Mg $ha^{-1}$ CC + 75% RFD and B3. 10 Mg $ha^{-1}$ CC + 50% RFD in three replications. Data were analyzed in variance at 5% significance level, and HSD test at 5% significance level. The parameters were ADF, NDF, crude Protein, crude fiber, cellulose, hemicellulose, lignin, silicates, plant height, maximum tillering, rice yield. The results showed that CC + RFD did not show different effects both on the growth and rice yield. Production of the highest forage obtained from Pandan Wangi rice crop reached 7,17 Mg $ha^{-1}$. Crude protein as much as 9.83% and 13,99%, crude fiber amounted to 18,31% and 20,15%, the rice yield as many as 6,29 Mg $ha^{-1}$; 4,21 Mg $ha^{-1}$ for Pandan Wangi and Cisokan respectively.

*Keywords: C.odorata compost, Pandan Wangi, Cisokan, ruminants forage*

## 1.Introduction

For thousands of years, rice has been a staple food source for many Asian countries like Indonesia, China, Japan and India. It's wonderfully versatile and is used as a base for many dishes from curries and stir-fries, to sushi and even puddings! A grain of rice is a seed from a special kind of grass called *Oryza sativa*. This grass needs lots of rain as it grows, and then dry conditions before it is harvested. Rice is grown in water-logged fields known as 'rice paddies' across Asia, but also in a few European countries, like Italy and Spain. Once the rice is harvested, the grains are shaken from the grasses, and their rough brown husks removed. Generally every region in Indonesia, especially in West Sumatra, paddy fields spread out evenly providers everywhere. Demand for rice in Indonesia continues to increase every year, simultaneously with the increasing population. Likewise, in addition, people also need a rice protein supplied from ruminant origin meat is maintained. Until 2016, Indonesia still imports of ruminants such as cattle and rice. However the tendency imports also decreased in connection with the intensification of land use as source provider of rice yield and cattle feed.

In addition to rice, the Indonesian country also imports beef, in 2012 total beef imported Indonesia reached 40 338 tonnes

worth US $ 156.138 million. Total costs incurred for the import of rice and cattle amounted to 1101.738 million US $ or the equivalent of 11.01 trillion rupiah (*Redaksi PI., 2013;* http://finance.detik.com/read/2013/02/04/075031/2160062/4/selain-daging-ini-bahan-pangan-yang-dibeli-ri-dari-luar-negeri?f991104topnews); (Statistika, 2016). This proves that Indonesia still has not sovereigned to food and meat.

Indonesia mostly farmers also raise cattle. The big problem for farmers is to provide productive grasslands, so that available forage needs of cattle. In fact pasture land has undergone a massive conversion, among others; for the construction of complex industrial factories, housing and some other goals. So the pasture available was be very limited. May not be planted wetland grasses by farmers, but likely to cultivate paddy rice also take advantage of young rice crops to be used as fodder.

There is an interesting case of rice cultivation activities, namely the fresh forage can be cut and without disturbing the rice yield. However there are some things that must be considered, so that the production of forage and grain yields remain high. If cuts were made early, it will be obtained the small amount of forage, but if the cut will be done lately, this would be concerned obtaining harvests decline, so farmers failed to harvest. Therefore, it needs the right time in an effort to cut forage in rice cultivation. Forage fodder is grass forage or that have nutritional adequacy rate appropriate for ruminants, not all can be categorized forage grass fodder. For that farmers need to grow their own grass forages superior categorized as such. Some types of this forage was from Indonesia and many are imported from abroad and developed in Indonesia (Jamilah & Helmawati, 2015); (http://kesehatan-ternak.blogspot.com/2013/02/hmt-hijauan-pakan-ternak.html, 2015).

In addition to caring moment precise cutting, the primary need to live rice crops also should drawn attention. Fertilizer right should be carried out, among others, can be either organic manure and fertilizers. (Jamilah, Adrinal, Khatib, & Nusyirwan, 2011) reported that administration *C.odorata* compost can improve nutrient uptake, growth and development of paddy rice roots. Integration cultivation pattern is considered more efficient, and can optimize the limited wetland. Need to know the quality of forage, milled rice produced by cutting the beginning of flower primordia on two varieties of rice plants from application of *C.odorata* compost accompanied by artificial fertilizers in paddy fields.

The research objective is to get fodder and higher rice yield in the cultivation of rice and cattle integration of the two rice varieties by *C.odorata* compost and fertilizers.

## 2. Methodology

The experiments was conducted for five months started in June 2015 through October 2015 in the paddy field farmer in the Padang city, the type of soil is Ultisol. Varieties tested were Pandan Wangi and Cisokan. The experiment was arranged in the split plot design. The main plot there were 2 levels cutting rice forage, which was not cut (Po) and cut as high as 15 cm of the soil surface at the beginning of flower primordia (+ 47 hst) (P1). The subplot was *C.odorata* compost (CC) combined with recommendation fertilizer dose (RFD) consisting of 3 levels; B1. 5 Mg $ha^{-1}$ CC + 100% RFD; B2. 7.5 Mg $ha^{-1}$ CC + 75% RFD and B3. 10 Mg $ha^{-1}$ CC + 50% RFD in three replications. Data were analyzed in variance at 5% significance level, and HSD test at 5% significance level. Recommendation fertilizer dose as 100 kg $ha^{-1}$ urea + 50 kg $ha^{-1}$ ZA, 150 kg $ha^{-1}$ SP36 and 100 kg $ha^{-1}$ KCl. Experiments conducted at SRI paddy in the pattern, 2 seeds in 1 planting hole, muddy, until the plant enters the flower primordia, a

spacing of 25 x 25 cm, and each plot size 2 x 2 m.

The parameters were ADF, NDF, crude Protein, crude fiber, cellulose, hemicellulose, lignin, silicates, plant height, maximum tillering, rice yield. Experimental data were analyzed using the F test 5% significance level, and conducted a further test HSD significance level of 5%, if the treatment significantly. The results of the analysis in the form of nutrient content in *C.odorata* Compost, nutrient content of forage fodder, not analyzed statistically, but compiled in table only.

## 3. Results and Discussion

Effect of fertilization on growth and yield of dry grain harvest is presented in Table 1. The interaction between fertilization and cutting can be seen in the production of dry grain harvest good varieties of rice Cisokan or at Pandan Wangi. Effect of fertilizer F2 (7.5 Mg ha$^{-1}$ *C.odorata* compost + 75% of artificial fertilizers) influenced the plant height and yield of rice grain. Tillers per clum was higher for PandanWangi than Cisokan. Yield of Cisokan had not reduced significantly by cutting but decrease to Pandan Wangi. The lower dose of organic fertilizer or higher amounts of artificial fertilizers, the plants grow better.

This was because artificial fertilizer as a fertilizer that is easily soluble and available, able to provide nutrients than organic fertilizers. As explained by (K. Mengel, Kirkby, Kosegarten, & Appel, 2001); (Weil & C.Brady, 2016) many macro nutrient elements needed by plants to produce component parts of vegetative and generative plant. Therefore, the availability of elements of N, P and K were quite decisive outcome dry grain harvest. Fragrant Rice Cisokan and GKP together produce more than 6 Mg ha$^{-1}$. Even at Cisokan that cut to get forage will not give worst effect to yield. According to (Mengel, 1995) that nutrient uptake per acre increases rapidly from the 4 leaf stage to just prior to tasseling, and then stays at high levels until after pollination. During this period the crop is growing very rapidly and the demand for nutrients to support that growth is high.

Tabel 1. Plant height, maximum tillering and production of dry grain rice harvest Cisokan and Pandan Wangi.

| Fertilizer | Plant height (cm) | | Maximum tillering per clump | | Yield of rice grain per plot (kg) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Cisokan | | Pandan Wangi | |
| | Cisokan | Pandan Wangi | Cisokan | Pandan Wangi | P0 | P1 | P0 | P1 |
| B1 | 81.50aB | 76.33bAB | 29.67 bA | 31.33 aA | 2.63 | 2.18 | 2,78a | 1,67b |
| B2 | 86.00aA | 73.00bB | 25.83 b B | 30.50 aA | 2.62 | 2.62 | 2,30a | 1,67b |
| B3 | 80.50aB | 78.50bA | 24.83 bB | 26.83 aB | 2.37 | 2.75 | 2,40a | 1,72b |
| average | 82.67a | 75.94b | | | 2.54 | 2.52 | 2,49a | 1,68b |
| average Mg /ha | | | | | 6,35 a | 6,29 a | 6,24a | 4,21b |
| CVA (%) | 3,01 | | | | | 15,03 | | 17,52 |
| CVB (%) | 6,08 | | | | | **9,27** | | 11,82 |

The numbers followed by the same capital letter in the same column and numbers followed by the same small letters on the same line are not significantly different according HSD 5% significance level.

Explanation: B1, 5 t ha$^{-1}$ *C.odorata* compost + 100% fertilizer; B2; 7,5 t ha$^{-1}$ *C.odorata* compost + 75 fertilizer; B3. 10 t ha$^{-1}$ *C.odorata* compost + 50% fertilizer; P0, not cut dan P1, cut at primordia age phase (47 days after planting.)

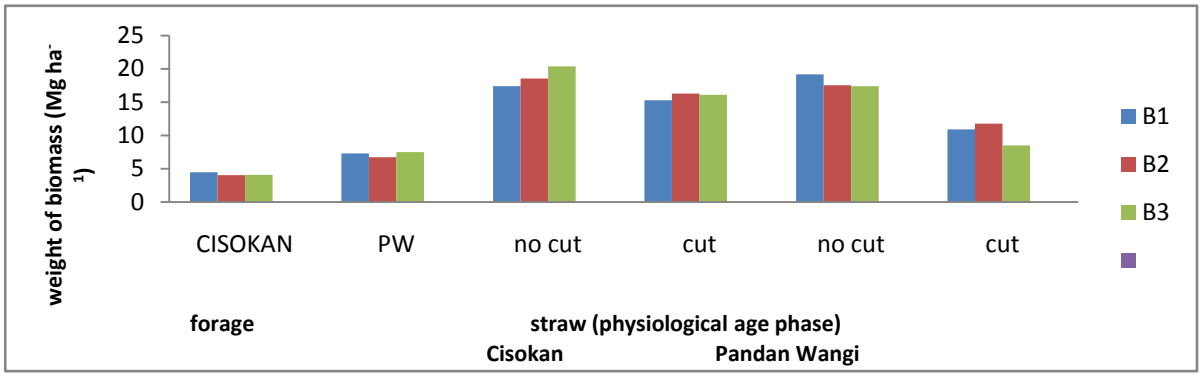


Figure 1. Effect of *C.odorata* compost + fertilizer to forage and straw weight of Cisokan and Pandan Wangi

2. Production and nutritional value of forage.

Giving compost and fertilizers showed no difference in the results of forage (Figure 1). Broadly speaking ruminant feed could be divided into two namely feed and feed reinforcing fibers, this fiber feed them grass and the amplifier is consentrate. Forage or Animal Feed should be cut at the right age, because if they are too old forage then the quality will be worse.

Pandan Wangi produced forage higher than Cisokan varieties. The results of the high forage is significantly affected by the maximum saplings in each variety. Plants that have a high maximum tillering forage will produce higher as well. Organic *C.odorata* compost gave effect longer and can improve soil fertility is inherently slow. Fertilizer is available and can be used again by the crops in the next growing season. It has been described by Brady (1984); Nyakpa *et al* (1988); Hakim (1985) that organic fertilizer, a natural fertilizer that can improve the quality of physics, chemistry and soil biological. Compost typically provide a longer effect than fertilizer. Manure can decrease the negative effects of the provision of artificial fertilizers and pollution are given excessive. This is because the organic fertilizer has carboxyl and phenolic capable of fixing metal ions that pollute the environment.

The results of the animal feed analysis, showed that the organic matter content, ash and crude fiber crude protein is generally higher than grass. This is due to rice crops that applicicated by compost and fertilizers are optimal, so it will affect nutrition. The results of the analysis of the nutrient content forage was presented in Figure 2 and 3.

In general, organic matter content, crude protein (CP) and crude fiber (SK) varieties Cisokan higher than Pandan Wangi. The forage of rice crops more nutritional content than elephant grass had CP reached 8,03% and CF reached 39,09% (Antonius, 2009). Nutrient content is also generally higher than the nutrients in rice straw either on Cisokan and Pandan Wangi. Jamilah et al., (2015) proved generally crude protein content in rice straw is only 50% compared to the content of the forage harvested at primordia age phase. When compared with the results of research (Sutardi *et al*., 1982; Zulbardi *et al*.,1983; Sitorus, 1989; Jackson, 1977) proving that the protein content of rice straw varies between 3-5%.

However, when compared to the crude protein and Crude Fiber contained in rice straw Cisokan and Pandan Wangi (Figure 2 and 3), then the quality of the straw was much lower than the grass, although the crude fiber content was still lower than the grass.

When compared with Antonius report (2009) that the dry matter content physiologically mature straw reaches 44.88%; 4.5% crude protein; 30,31% crude fiber. Rice bran contains 10.61% crude protein; 14.13% crude fiber and 91.31% dry matter. For

elephant grass, 20.23% dry matter; 8.71% crude protein and 28.35% crude fiber.

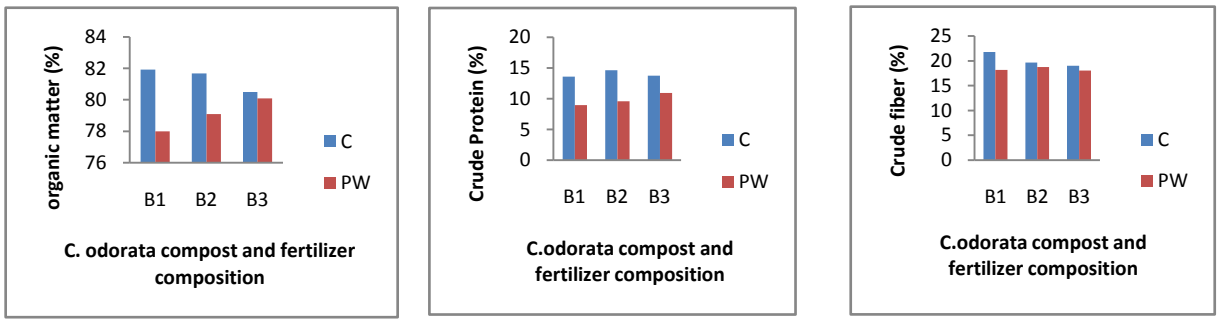


Figure 2. Organic matter content (BO), crude protein (CP) and Crude Fiber (CF) on two varieties of rice which be cut when primordia age phase

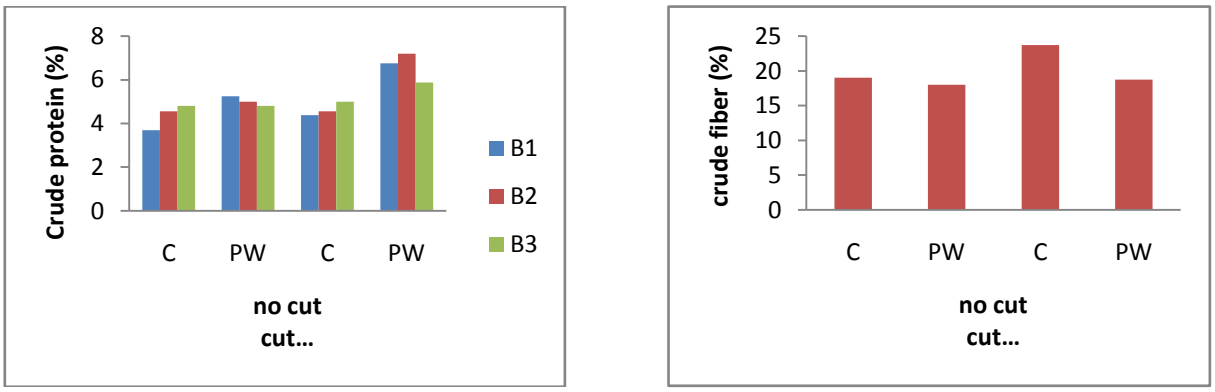


Figure 3. Crude protein (CP) and Crude Fiber (CF) of Cisokan (C) and Pandan Wangi (PW) of rice straw when physiological age phase

When compared to the crude protein of the rice plants were cut 47 days after planting (primordia age phase), with elephant grass plants, the rice plants are superior quality. Zulbardi (2000) showed that the levels of crude protein grass good quality ranges from 9%, while Zubaidah (2008) found ranged from 8.08 to 10.86%, still lower than the crude protein content of rice plant was cut 47 days after planting. Crude protein in forage origin of the rice plants are cut when the plant 47 HST containing 2-fold compared to the hay crop reached a mature age physiologically.

Ruminant livestock forage require materials with a value of at least 50-55% digestibility and crude protein (CP) approximately 8% (Thaliba, et al., 2000). When compared with the results of forage cultivation pattern integration of cattle and rice, the rice plants when the initial cut into the primordial interest (47 HST), PK reached 14% in plants Cisokan, and Pandan Wangi CP reached 10.94%, much higher quality to be used as ruminant feed.

The ash content of the mineral material content which does not include constituent organic material in the plant. The ash content is derived from the mineal elements absorbed by plants from the soil like elements K, Ca, Mg, Fe, Mn, Cu, P, and there is mention N also belong to it. The content of phosphorus and calcium are available from rice straw is also low. In addition to the low protein

content, rice straw also has a value of dry matter and organic matter is low, the row between 34-52% and 42-59% (Winugroho et al., 1983). This led to the low digestibility of dry matter intake low ability, which is only 2% of body weight (Jackson, 1977; Utomo et al., 1998). When compared with the results of research Zubaidah (2008), proving that the ash content in the elephant grass plants from 8.24 to 12.48% and Zulbardi (2000) reported at 10.29%. Production of organic matter, crude fiber, ash and phosphate were higher in plants fertilized with F1 treatment (5 Mg ha$^{-1}$ compost fertilizer *C.odorata* + 100% fertilizer) compared to treatment plants that are fertilized F2 and F3 (Figure 4).

Cisokan rice plants and Pandan Wangi cut early when entering the flower primordia, able to produce up to 2.9 Mg of dry matter per hectare, organic material up to 2.4 Mg ha$^{-1}$. Varieties Cisokan more response from application of fertilizer F1, F2 and F3 compared, while Pandan Wangi more responses if given fertilizer F3. Cisokan on rice plants, in general production of the highest nutrient fertilization treatment results F1 (5 Mg ha$^{-1}$ *C.odorata* compost + 100% artificial fertilizers), while Pandan Wangi higher nutrient content of F3 treatment outcome (10 Mg ha$^{-1}$ compost *C.odorata* + 50% artificial fertilizers). Striking differences from the production of nutrients produced from forage varieties of different origin, caused also by the different plant ages. Cisokan an old plants longer than Pandan Wangi, so the ability of the recovery is more adequate than Pandan Wangi in producing top stover and establishment of rice plants flowering in time.

In Table 2 and 3, featuring content of NDF, ADF, lignin, silica, cellulose and hemicellulose in green forage. Van Soest analysis results indicate that rice Pandan Wangi values ADF and cellulose, higher than rice and Cempo Cisokan Red. Provision of fertilizer 5 t ha-1 compost C.odorata + 100% artificial fertilizers produce high levels of ADF and cellulose lower than if the compost increased the proportion of up to 10 Mg ha$^{-1}$ of all varieties of rice. But conversely, the higher the composting C.odorata, hemicellulose content of silica and getting lower.

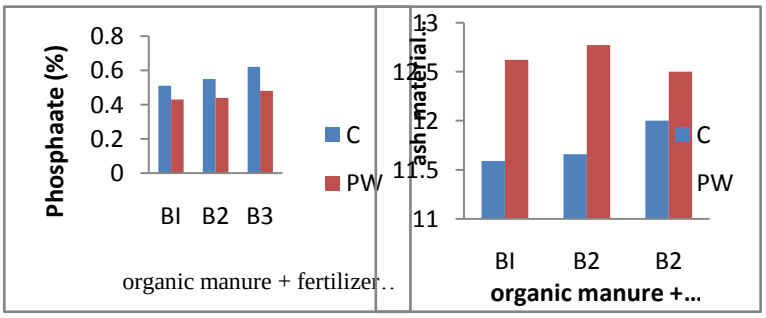


Figure 4. Phosphate a and Ash material in 2 rice crop varieties at primordial and hysiological age phase (%).

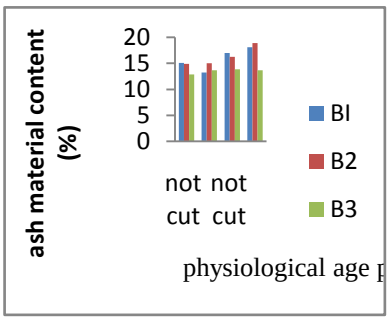


Table 2. ADF, NDF, Lignin and Silica of forage of Cisokan and Pandan Wangi at primordia age phase

| Fertilizer | ADF (%) | | NDF (%) | | Lignin | | Silica | |
|---|---|---|---|---|---|---|---|---|
| | Cisokan | Pandan Wangi | Cisokan | Pandan Wangi | Cisokan | Pandan Wangi | Cisokan | Pandan Wangi |
| B1 | 35.96 | 33.25 | 70.79 | 59.38 | 5.23 | 2.50 | 5.19 | 6.37 |
| B2 | 37.12 | 40.30 | 69.17 | 64.70 | 9.75 | 3.37 | 5.86 | 6.98 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| B3 | 35.51 | 43.69 | 61.84 | 66.18 | 3.43 | 5.88 | 6.28 | 5.67 |

Table 3. Cellulose and hemicellulose of forage at primordia age phase

| Fertilizer | cellulose (%) | | hemicellulose (%) | |
|---|---|---|---|---|
| | Cisokan | Pandan Wangi | Cisokan | Pandan Wangi |
| B1 | 25.24 | 24.38 | 34.83 | 26.13 |
| B2 | 21.51 | 29.96 | 32.05 | 24.40 |
| B3 | 25.63 | 28.75 | 26.33 | 22.49 |

The content of cellulose in rice Pandan Wangi, higher indicates that the feed that comes of this rice has a better digestibility than other types of rice. When compared with the nutrient content of grass Ruzi developed by Hutasoit et al., (2009) prove that the NDF and ADF on grass Ruzi (*Brachiaria ruziniensis*) used is relatively young so it can deliver good nutrition, such as protein content is high (14%) , 50-61% NDF and ADF content ranges from 35-40% (Hutasoit, et.al., 2009).

The higher levels of silica in the feed will also complicate the digestibility for ruminants. Pandan Wangi have a silica content higher than Cisokan. The effect of compost is also something to do with the content of silica in the rice straw, the lower the composting, the higher silica content. According to (Laboratorium Team, 2013) if a low protein content causes the digestibility of only 40% result in a lower dry matter intake (less than 2% weight of livestock). It was clear, without adding concentrate was not possible to increase the production of livestock, may even be able to reduce the production. Another problem affecting the quality of hay is the high content of lignin and silica causing so low digestibility.

## Conclusion

*C.odorata* Compost + FDR did not show different effects both on the growth and rice yield. Production of the highest forage obtained from Pandan Wangi rice crop reached 7.17 Mg $ha^{-1}$. Crude protein as much as 9.83% and 13.99%, crude fiber amounted to 18.31% and 20.15%, the rice yield as many as 6.29 Mg $ha^{-1}$; 4.21 Mg $ha^{-1}$ by Pandan Wangi and Cisokan respectively.

## Acknowledgement

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## References

Afandi, R., 2002. Ilmu Kesuburan Tanah. Yogyakarta: Penerbit Kanisus.

Bachtiar, E., 2006. Soil Science. Medan: Fakultas Pertanian USU.

Buckman NC, Brady HO. 1987. Soil Science. Jakarta: PT. Bharata Karya Aksara.

Beranda Jembatan Inovasi Teknologi. 2014. 10 items food Indonesia still imports. http://beranda.miti.or.id/10-bahan-pangan-indonesia-masih-impor/, akses 4 April 2014.

BPS.2013. Indonesia in Figures. Central Bureau of Statistics

Haryanto, B. 2003. Jerami padi fermentasi sebagai ransum dasar ruminansia. News Agricultural Research and Development. 25 (3): 1–2.

Hendra Z. 2006. Study of Carbon And Nitrogen Ratio Of Onggok And Urea In Production By biopesticide *Bacillus thuringiensis* Subsp.

*Israelensis*. Faculty of Agricultural Technology, Bogor Agricultural University.

http://www.artikelbagus.com/2012/01/sekam-padi-kulit-gabah.html#ixzz3i6F3W6Md formed on rice contain carbohydrates dominant, 20.

http://kesehatan-ternak.blogspot.com/2013/02/hmt-hijauan-pakan-ternak.html. 2015. Livestock, Forage, Animal Feed. http://kesehatan-ternak.blogspot.com/2013/02/hmt-hijauan-pakan-ternak.html.

Hutasoit, Rianto, Juniar Sirait dan Simon P. Ginting. 2009 Technical instructions Cultivation and Utilization *Bachiaria ruziziensis* (Grass Ruzi) As Foliage Feeding Goats. Centre for Research and Development of Animal Husbandry Agency for Agricultural Research and Development Department of Agriculture.

Jamilah, Yohanes dan Widodo Haryoko. 2008. Residual effect of compost of *C.odorata* and economical Guano effort of manure made in for the crop of onion at marginal land; ground.Jurnal Embrio. Agriculture Faculty Tamansiswa University Padang ISSN N0. 2085-403X Vol.(I) No. 2: 63-73.

Jamilah, Adrinal, Khatib, I., & Nusyirwan. (2011). Waste Land Reclamation impacting Cement Raw Material Mine Through the Utilization of Organic Fertilizer In Situ To Improve Results Rice. In the National Seminar on the topic of Organic-Based Integrated Agricultural Development Toward Sustainable Agricultural Development (pp. 172–189).

Jamilah, & Helmawati. (2015). Study Analyse Farming Integrate Paddy Rice field and Livestock feed of Ruminansia Support Sovereignty of Food and Flesh In Face Of Economic Society of Asean 2015. In Seminar National of[is Readiness of Indonesia in face of Free Market [of] Asean Through Reinforcement Of Implementation of Corporate Healthy Governance (Vol. 3, pp. 254-266). Field: Library of National of RI.

Laboratorium, T. I. dan T. P. F. P. I. (2013). Knowledge of Food-Stuff Livestock. *CV NUtrisi Sejahtera* (Vol. 53). Bogor: CV Nutrisi Sejahtera. http://doi.org/10. 1017/CBO978 11074 15324.004

Mengel, D. (1995). Roots , Growth and Nutrient Uptake. Agronomy Department, Purdue University West Lafayette, IN 47907-1150 While.

Mengel, K., Kirkby, E. a., Kosegarten, H., & Appel, T. (2001). Principles of Plant Nutrition Edited by and, *5th*, 849 pp. http://doi.org/10.1007/978-94-010-1009-2.

Statistika, B. P. (2016). *Output Tabel Dinamis of Badan Pusat Statistika*. Indonesia. Retrieved from http://www.bps.go.id/mod/exportData/exportPDF.php.

Weil, R. R., & C.Brady, Ny. (2016). *the Nature and Properties of Soils. 15th edition*. *Constraints* (Fifteenth). Macmillan: Macmillan Publishing Company.

Zulbardi, M., A.R. Siregar dan I-W. Mathius. 1983. Rice straw with the corn and rice bran as food buffalo. Pros. Large Ruminant Scientific Meeting. Puslitbangnak, Bogor. Hlm 33-36.

# Effectiveness of Liquid Smoke to Control Mealybug on Papaya

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## Abstract

Mealybug (Paracoccusmarginatus) is an important pest that has a high destructive power on papaya. The objective of this study was to determine the effective concentration of liquid smoke to control mealybug on papaya. The study was conducted at the Laboratory of Pest and Disease Indonesian Tropical Fruit Research Institute, Solok in October 2016. The experiment was arranged in randomized complete design with five treatments and five replications, each replication consisted of 10 mealybugs. The treatment was concentration of liquid smoke, namely A = 0.05 ml / l, B = 0.1 ml / l, C = 0.2 ml / l, D = 0.4 ml / l and E = control. The mealybugsthatbe tested were the third instar of mealybugs which be collected from papaya plant. Variables were letal concentration (LC) 50, the percentage of mortality and the mortality rate of mealybug. The results showed that the effective concentration at 50% mortality (LC50), the use of liquid smoke on the first day was 0.79 ml / l, the second day of observation was 0.28 ml / l, while on the third day was obtained at a concentration of 0.08 ml / l. The highest percentage of mortality was obtained at a concentration 0.4 ml /l of liquid smoke. Liquid smoke was significant to mealybugs mortality up to 73.33%.The results showed that liquid smoke had potency as a botanical pesticide to control mealybug on papaya. The liquid smoke was effective to control mealybug which safe for environment, and low cost. 0.4 ml/l liquid smoke could kill 73.3% of mealybugs. The concentration less than 0.1 ml/l was not too significant to control mealybugs.

Keywords: liquid smoke, mortality, mealybug, pesticide

## 1. Introduction

Papaya mealybug, Paracoccusmarginatus (Hemiptera: Pseudococcidae), It was reported as a pest of papaya (Carica papaya L.) in St. Martin Island in the Caribbean in 1995, and by 2000 it had spread to 13 countries in the Caribbean, Florida in the United States, and six countries in Central and South America [1]. The mealybug caused significant yield losses not only in papaya but in more than 60 other crops, particularly horticultural species [2][3].

Mealybug (Paracoccusmarginatus) is an important pest that has a high destructive power on papaya. Terrible attacks have occurred at 2008 on farmers` land in Bogor [4][5]. The development of local natural enemy cannot compensate populations of P. margitus, especially during dry season therefore it can cause a heavy attack.

Mealybug attacks usually occurred during dry season. The farmers do controlling by hand or tools, spraying detergent and using insecticides which have imidacloprid active ingredients [6]. The controlling of this pest still using chemical pesticide so the cost is still high. Besides, wrong concentration creates new problem because it cause pesticide residues in fruit, while consumers want fruit free of pesticide residues. Botanical pesticide is an answer to the challenge. One of botanical pesticide is liquid smoke.

Liquid smoke is a mixture of the solution and a colloidal dispersion of wood smoke or coconut shell in the water were obtained from the pyrolysis of wood or a mixture of pure compounds. The using of liquid smoke as environmental bio-pesticide because it is easy to decompose, 1% liquid smoke was combined with waxing gave the best results in the maintaining the quality of papaya fruit [7]. Test of antimicrobial activity and molds showed that 1% liquid smoke could inhibit the growth of bacteria and molds [8]. Liquid smoke derived from coconut shell is a product that is easy to be obtained. This material has been declared safe to be used for preservation of food products [9]. Smoke of coconut shell is an ingredient in the manufacture of liquid smoke. It was used as a fumigant to control pest Rhyzoperthadominica in rice warehouse [10]. The coconut shell which not burning perfectly will generate smoke containing several kinds of compounds namely 4.13% fenol, 11.3% carbonyl, 10.2% acid compounds and polycyclic aromatic hydrocarbons [11]. Liquid smoke is used as a fungicide to control rot disease of pinus seed.

The objective of this study was to determine the effective concentration of liquid smoke to control mealybug on papaya.

## 2. MaterialandMethods

*2.1.Structure*

The study was conducted at Laboratory of Pest and Disease Indonesian Tropical Fruit Research Institute, Solok in October 2016.The experiment was arranged in randomized complete design with five treatments and five replications, each replication consisted of 10 mealybugs.The treatment was concentration of liquid smoke, namely A = 0.05 ml / l, B = 0.1 ml / l, C = 0.2 ml / l, D = 0.4 ml / l and E = control. The mealybugs which be tested were the third instar of mealybugs which collected from papaya plant. Before the research was started, the mealybugs were rearing and propagated in the papaya tree of 3 weeks old after planted for ± 1 month. The method to pesticide application in this study was by dyeing. The young leaves of papaya prepared as an artificial habitat of mealybugs were dipped into liquid smoke solution according to treatments and dried for 30 minutes. The leave stalks were coated by wet cotton then put into a petri dish. Furthermore 10 mealybugs were put into leaves and stored at room temperature.

The observation of liquid smoke effectiveness to control mealybug was done in every 2 days up to one week. During the observation, the addition of water on cotton must be done to keep the leavesfrimness.

The observations namely:

1. Percentage of mortality / death mealybug

The percentage was calculated by the following equation:

$$Pt = \frac{P0 - Pc}{100 - Pc} \times 100\%$$

Description:

Pt = percentage of death correcte

Po = percentage of death observed

Pc = percentage of death control

2. Test of LC 50.

This test was calculated by using probitanalysis with Minitab Statistical Software 14.

3. The rate of mortality of mealybug on some concentrations was calculated by the death of mealybug in every observation.

## 3. Result andDiscussion

*3.1.Percentage of Mortality*

The results showed that liquid smoke had potency as a botanical pesticide to control mealybug on papaya. Four concentrations of liquid smoke caused mortality higher than control about 2-3 times. Concentrations of liquid smoke among A (0.4 ml/l), B (0.2 ml/l), and C (0.1 ml/l) were not significant different, however concentration D (0.05 ml/l) was significant different to concentration A (0.4 ml/l). All treatments were significant different to control (Table 1).

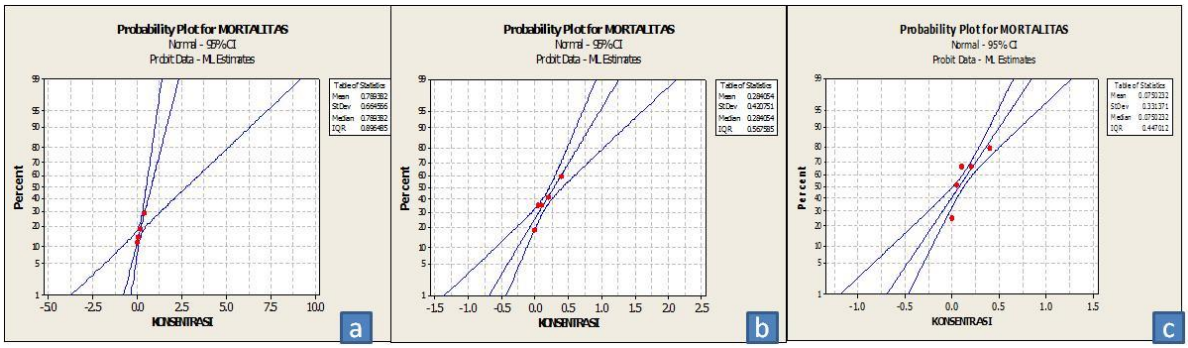


Fig. 1. Probit analysis of LC 50 on mealybug

Table 1. Percentage of Mortality on Some Concentrations at 6 Days After Application

| Treatment (Concentration of smoke liquid) | Percentage of Mortality (%) |
|---|---|
| 0.4 ml/l | 73.33[a] |
| 0.2 ml/l | 51.06[ab] |
| 0.1 ml/l | 46.66[b] |
| 0.05 ml/l | 35.56[b] |
| Control | 25.00[c] |

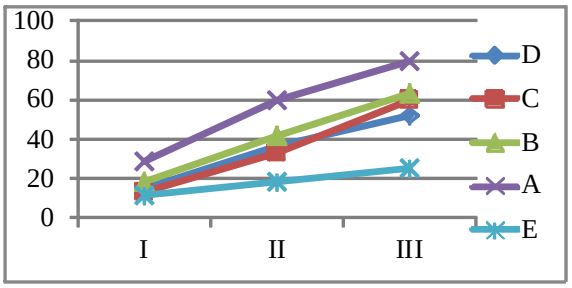


Fig. 2. The rate of mortality

The research showed that liquid smoke of coconut shell on 0.1 ml/l gave significant effect on mortality of mealybug (Paracoccusmarginatus). 0.4 ml/l concentration of liquid smoke could kill 73.33% of mealybugs, even the lowest concentration (0.05 ml/l) still could kill 35.56% of mealybugs. All concentrations were significantly different to control (Table 1). Data observation explained that the higher concentration of liquid smoke made the higher of mortality also. At the last day of observation, 0.1 ml/l liquid smoke able to kill 50% of mealybugs. This was low concentration which safe for environment, and also low cost.

*3.2.Test of LC 50*

Probit analysis showed that liquid smoke caused 50% mortality (LC-50) at the first day of observation at concentration 0.79 ml/l (Figure 1a), and the concentration which close LC-50 was 0.4 ml/l (treatment A). At the second day, 0.28 ml/l caused 50% mortality (Figure 1b), and the concentration which close LC-50 was 0.2 ml/l (treatment B). At the third day, the highest mortality for test of LC-50 was at 0.08 ml/l (Figure 1c), and the concentration that almost similar to this concentration was 0.1 ml/l (treatment C).

*3.3. The rate of mortality*

The observation on mortality of mealybugs with some concentrations of liquid smoke showed that liquid smoke was significant (Fig. 2). The daily mortality increased at the second day, 0.1 ml/l could kill 50% of mealybugs. Mortality increased time by time.

There were some benefits of liquid smoke; which could be used as a botanical pesticide. The liquid smoke of coconut shell contains phenolic compounds which kill pests, and acid compounds. Liquid smoke of coconut shell has antifeedant bioactive compounds. These compounds are needed by plants to protect themselves from pests,

microbes, and other organisms. These bioactive cannot kill the pests directly but as an inhibitor eating of pests. Automatically pests will not eat the plants. [12]added that liquid smoke reduced the damage of termites and wood processing. Liquid smoke has function as an contact insecticide that contains fenol/eugenol so it easy to absorb through the surface of larva body [13]. Contact insecticide will enter to the body through the larva cuticle, if the skin has a contact with insectiside then the insecticide molecul will enter to larva body. Along with the addition time, the accumulation of insecticide that enter larva body can caused death [14] .

## Conclusion

The liquid smoke was effective to control mealybug which is safe for environment, and low cost. 0.4 ml/l liquid smoke could kill 73.3% of mealybugs. The concentration less than 0.1 ml/l was not too significant to control mealybugs.

## References


[1] Muniappan, R., Papaya mealybug, new invader in Asia. IAPPS Newsl. 2009.

[2] Meyerdirk, D.E., Kauffman, W.C., Status on the development of a biological control program for Paracoccusmarginatus Williams, papaya mealybug, in the Caribbean. In: Paper Presented at IV International Scientific Seminar of Plant Health, Veradero, Cuba. (Abstract). 2001.

[3] Miller, D.R., Miller, G.L.,.Redescription of Paracoccusmarginatus Williams and Granara de Willink (Hemiptera: Coccoidea: seudococcidae), including descriptions of the immature stages and adult male. Proc. Entomol. Soc. Wash. 104, 1e23. 2002.

[4] Muniappan R, BM Shepard, GW Watson, GR Carner, D Sartiami, ARauf, & MD Hammig. First report of the papaya mealybug, Paracoccusmarginatus (Hemiptera: Pseudococcidae), in Indonesia and India. J. Agric. Urban Entomol. 25: 37-40. 2008.

[5] Sifa A, D. Prijono& A. Rauf. Keefektifan tiga jenis insektisida nabati terhadap kutu putih papaya Paracoccus marginatus dan keamanannya terhadap larva kumbang predator Curinuscoeruleus. J. HPT Tropika. 13(2): 124 – 132. 2013.

[6] Sartiami D, Dadang, Anwar R, & Harahap IS. Persebaran hama baru Paracoccus marginatus di ProvinsiJawa Barat, Banten, dan DKI Jakarta. Hlm 453-462 dalam: Prosiding Seminar NasionalPerlindunganTanaman. Bogor, 5-6 Agustus 2009. Pusat Kajian Pengendalian Hama Terpadu IPB, Bogor. 2009.

[7] Oramahi, HA, Diba, F, Tavita, GE, & Wahyuni, R, Penggunaan asap cair dari tandan kosong kelapa sawit (TKKS) dalam penekanan perkembangan jamur Schizopyllum commune. Jurnal Tengkawang 1 (2): 51-56. 2011.

[8] Budijanto, S, Hasbullah, R, Prabawati, S, Setyadjit, Sukarno &Zuraida, I. Pengembangan dan pemanfaatan asap cair tempurung kelapa untuk pengawetan produk buah–buahan. Abstrak Laporan KKP3T. 2007.

[9] Budijanto, S, Hasbullah, R, Prabawati, S, Setyadjit, Sukarno &Zuraida, I. Identifikasi dan uji keamanan asap cair tempurung kelapa untuk produk pangan. Pascapanen 5(1) : 32-40. 2008.

[10] Aryawan A.A.K., B.T Rahardjo, L.P Astuti. Potensi asap pembakaran tempurung kelapa dalam pengendalian hama Rhyzoperthadominica F. (Coleoptera: Bostrichidae) pada gabah dalam simpanan. J. HPT 1(1) : 6 – 11. 2013.

[11] Nurhasanah, E. Perancangan Alat untuk Membuat Asap Cair dari Tempurung Kelapa dan Karakterisasinya (Tesis). Program Studi Kimia Pasca Sarjana Institut Teknologi Bandung. 2008.

[12] Wijayanto and EndangWarih. Kajian Potensi Asap Cair dalam Mengendalikan Ulat Krop Kubis C. Pavonana. Unsud. 2008.

[13] Wilbraham AC, Matta MS. Pengamtar Kimia Organik dan Hayati. Suminar (Penerjemah). Terjemahan dari Introduction to Organic and Biological Chemistry. Bandung. ITB. 1992.

[14] Wudianto R. Petunjuk penggunaan pestisida. Jakarta. Penebar Swadaya. 1998.

# Study on the Manufacture of Nuggets from Natural Rubber Seed *(HeveaBrasilinsis Mull. Arg)*

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## Abstract

The research on the study of manufacture of nuggets from natural rubber seed has been carried out by completey randomized design (CRD) with 2 replications, the first factor is the long boiling (L) is: $L_1$:5 minute, $L_2$:10 minute, $L_3$:15 minute, $L_4$:20 minute. The second factor is the addition of wheat flour (P), namely: $T_1$:20 %, $T_2$:30%, $T_3$:40 %, dan $T_4$: 50%. Parameters observed included: Protein contet, ash contet, organoleptic aroma, texture and taste. Statistical analysis of the results obtained, that the boiling time effect is not real ($P<0.05$) to protein contet, ash contet, organoleptic aroma, texture and taste. Extra flour gives no real influence ($P<0.05$) to protein contet, ash contet, organoleptic aroma, texture and taste.

Keywords: rubber sheed, boiling, addition of wheat flour

## 1. Introduction

Innovation on food processing is needed at this time. High activity led to the public demand for fast food and choice, has no nutritional value and safe for consumption. Nugget is one of the food is fast food that has a high protein content and has a flavor that varies.

Nugget on the market usual meat-based either chicken or beef.Nugget of this type is already popular among our people and also has a good flavor and are very popular with young children. But the price of meat-based nugget is quite expensive, so not all people can enjoy it. Therefore, the authors took the initiative to make the nuggets from raw material rubber seeds, which have a protein content that is not less important than the chicken (Elsawati, 2014).

Rubber is a product of the estate, which until now used the sap and trunks. Rubber seed not fully utilized, as well as plant seeds course, that was ten years old may be used as seed because it comes of age and desired criteria wasted the rest is left without any utilization (Muchtadi, 1985).

## 2. MaterialandMethods

### *2.1.Materials*

Materials used in this study are as follows: rubber seeds, water, eggs, garlic, starch, flour, pepper, salt, flour and sugar crumb.

### *2.2. Research methods*

The research method was conducted using completely randomized design (CRD) factorial consisting of two factors, namely:
Factor I: Boiling Lama (L) which consists of 4 levels, namely:
L1 = 5 minutes
L2 = 10 minutes
L3 = 15 minutes
L4 = 20 minutes
Factor II: The addition of flour (T), which consists of 4 levels, namely:
T1 = 20%, T2 = 30%, T3 = 40%, T4 = 50%
The number of combined treatment (Tc) is 4 x 4 = 16, then the number of replications (n) is as follows:

Tc (n-1) ≥ 15, 16 (n-1) ≥ 15, 16 n-16 ≥ 15,n16
n ≥ 31
n ≥ 1.937 ..... rounded to n = 2
then for thorough research, conducted replications of 2 (two) times.

*2.3 Model Design of Experiments*

This research was conducted with a completely randomized design (CRD) factorial model:

$\tilde{Y}ijk = \mu + \alpha i + \beta j + (\alpha\beta)\, ij + \varepsilon ijk$

Where :

$\tilde{Y}ijk$: Observations of a factor L from level to - i and T factor in the extent to - j replicated to - K.

μ Effect middle value.

αi: Effect of L factors on the level to - i.

βj: Effects of the extent to factor T - j.

(Aβ) ij: interaction Effect factor L at the level to - i and T factor in the extent to - j.

εijk: Effects remainder of factor L at the level to - i and T factor in the extent to - j in replicated to - k.

*2.4. Experimental*

1. Seed rubber cleaned of dirt.
2. Seed rubber peeled with ash and leaves will be discarded.
3. Next direndaman for 48 hours, changing the water 2 times.
4. Then drained and boiled rubber seed according to treatment.
5. Seeds of rubber that has been boiled mixed with tapioca flour, wheat flour according to treatment, salt, pepper, sugar and garlic.
6. Kneading mixed until homogeneous, then the form of nuggets.
7. Pengkukusan nuggets up to 10 minutes, then rubbed with a whisk eggs until uniform and jerky in the flour crumb.
8. Fried nuggets, after mature analysis.

Testing parameters were ash content, protein content, organoleptic texture, flavor and aroma.

## 3. Result andDiscussion

From the research and statistical tests, generally indicates that the comparison of boiling time effect on the observed parameters. From the average data comparison observation influence the addition of wheat flour to the parameter;

*3.1. Protein levels*

The effect of boiling time on protein levels comparison of long boiling effect is highly significant (P <0.01) the protein content.From Table 7 it can be seen that the L1 significantly different from L2, and highly significant with L3 and L4. Significantly different L2 and L3 to L4 highly significant.L3 to L4 highly significant. The protein content is highest in L4 treatment (20 minutes) that is equal to 17,169% and the lowest in treatment L1 (5 minutes) 15 249%. To more clearly can be seen in Fig.1.

From Fig.1 that the longer boiling the protein content increased. This is because at the time of boiling fat contained in Nugget soluble in hot cooking water, which causes the amount of fat contained in Nugget reduced and dissolved in hot water, with a reduced amount of fat contained in the Protein levels Nugget resulted in increased Nugget. So that the protein content will increase with the longer boiling (Swaminathan.1974).

Effect of the Addition of Wheat Flour Protein Levels

From the analysis of variance Appendix 1 can be seen that the addition of wheat flour provides highly significant effect (p <0.01) the protein content.

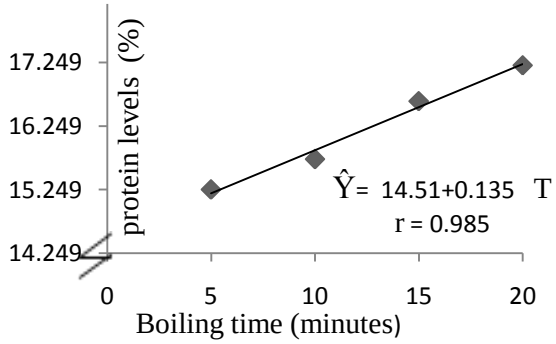


Fig. 1 Graph of boiling time on protein levels

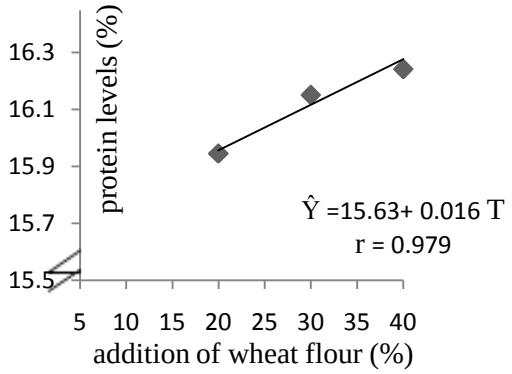


Fig.2 Relationship addition to the protein content of wheat flour

The T1 had no significant T2, highly significant with T3 and T4. The highest protein content was found in the T4 treatment (50%) 16 448% and the lowest at T1 (20) 15 945%. The higher the addition of wheat flour were added in the manufacture of rubber seed nuggets then occur and to more clearly seen in Fig.2.

It can be seen that the higher the addition of wheat flour, the protein content will increase. This is caused because the flour has a high protein content so that the content of protein in Nugget increased, so that the protein content increased by (Haryanto 1992) Wheat flour is flour or fine powder derived from the ears of corn, and is used as the base material cake maker , noodles, bread and other foodstuffs. Wheat flour also contains starch, protein in the form of gluten, which play a role in determining the viscosity of foods made from wheat.

Interaction Effect of boiling timeand additions to the protein content of wheat flour

From the list of variance Appendix 1 can be seen that the interaction between the boiling time and the addition of wheat flour no significant different effect (P> 0.05) to the protein content of the seed rubber nuggets produced so LSR test was discontinued.

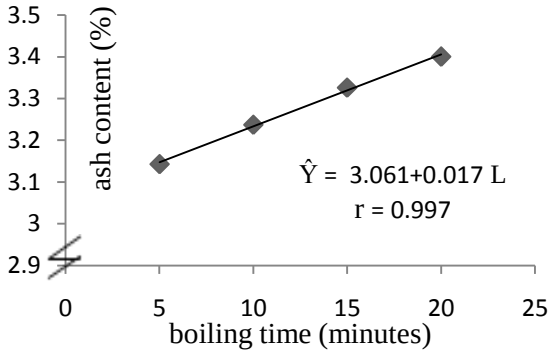


Fig.3 Graph of boiling time on the ash content

*3.2. Ash content*

The effect comparison of boiling time onash content

It can be seen that the significantly different L1 L2, and highly significant with L3 and L4. Significantly different L2 and L3 to L4 highly significant.L3 to L4 highly significant. The ash content is highest in L4 treatment (20 minutes) that is equal to 3,509% and the lowest in treatment L1 (5 min) 3.143%. To more clearly can be seen in Fig.3.

The ash content the longer boiling time, the ash content of the resulting increase. This is because the longer boiling, the more water is evaporated in the material, thereby increasing ash content by weight of dry ingredients, or the increasing weight of the wet material. According to (Sudarmaji at al, 1997) that the ash content varies with the type of materials, means of ashing, time and temperature are used when processing takes place. and the higher the temperature used in the boiling process will affect the ash content, water content is removed from the material increases.

Effect of addition of wheat flour on ash content

From the analysis of variance Appendix 2 can be seen that the addition of wheat flour provides highly significant effect (p <0.01) the protein content. the differences of each level.

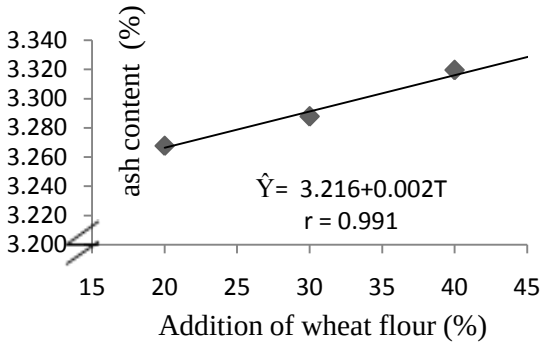


Fig.4 Graph of wheat flour on ash content

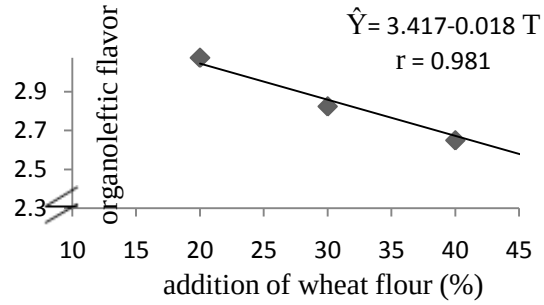


Fig.5 Graph of addition wheat flour on organoleptic flavour

It can be seen that the T1 had no significant T2, highly significant with T3 and T4. Abu highest levels found in the T4 treatment (50%) 3,340% and the lowest at T1 (20) 3,268%. The higher the addition of wheat flour were added in the manufacture of rubber seed nuggets then occur and to more clearly seen in Fig.4.

It can be seen that the higher the addition of wheat flour increased ash content. This is because the composition of the nuggets of rubber seeds also contain minerals - minerals. The mineral content of wheat flour is the one that causes the ash content in the nugget increased if the flour is added more and more. It is appropriate (Rabah and abdila, 2012) states that the mineral elements, also known as inorganic substance or the ash content, the flour has a diverse mineral content. The mineral content contained in 100 grams of wheat flour: water 12 g, carbohydrates 74.5 g, fat 11 g, calcium 1.3 mg, phosphorus 106 mg, iron 1.2 mg.

Interaction Effect of Boiling and Additions Old Flour against Ash Content

From the list of the varieties dik Appendix 2 can be seen that the interaction between the boiling time and the addition of wheat flour tidaknyata different effect (P> 0.05) to the ash content nugget rubber seed produced so LSR test was discontinued.

*3.3. Organoleptic flavor*

Boiling time influenced the flavor from the list of variance Appendix 3 shows that the boiling time effect was not significant (P> 0.05) to the sense nugget rubber seed that produced so that further testing was discontinued.

Effect of Wheat Flour Addition on Flavor

From the analysis of variance attachment 3 can be seen that the addition of wheat flour provides highly significant effect (P <0.01) in the flavor. Differences respectively.

It can be seen that the T1 had no significant T2, highly significant with T3 and T4. Organoleptic taste value highest in T1 treatment (20%) 3,075% and the lowest was T4 (50%) 2,513%. The higher the addition of wheat flour were added in the manufacture of rubber seed nuggets then occur and to more clearly seen in Fig.5.

From Fig. 5 it can be seen that the higher the addition of flour then it seems to decline. This happens because the higher the flour is added to the manufacturing of rubber seed nugget, the more the addition of flour. Causing fusion between the material forming other less balanced blend that is less balanced led to a sense that produced decreases. This is consistent with the statement (Deman 1997) which states that the flavor is a combination of properties - characteristic of materials that produce a sensation (stimulus). The decline in the organoleptic quality wheat flour with the addition of more and more due to the increase of the flour in nuggets cause taste

becomes less tasty. the higher the rate of addition of wheat flour led to the combination of materials forming other less balanced. Less balanced blend of flavors produced causing decreases.

*3.4. Organoleptic taste*

From the list of variance Appendix 3 shows that the interaction between the boiling time and the addition of wheat flour non significant different effect (P> 0.05) to the value of organoleptic taste nugget rubber seed produced so LSR test was discontinued.

*3.5. Texture Appearance*

Effect of long boiling on the texture of rubber seed

From the analysis of variance attachment 4 can be seen that the boiling time provides highly significant effect (P <0.01) in the texture of rubber seed nuggets.

The significantly different L1 L2, and highly significant with L3 and L4. Significantly different L2 and L3 to L4 highly significant. L3 to L4 highly significant. Organoleptic texture highest in L4 treatment (20 minutes) 2,638% and the lowest in treatment L1 (5 min) 2,225%. To more clearly can be seen in Fig. 6.

The longer boiling time led to the increasing value of the texture of the nugget rubber seeds. This relates to the water content of the nugget increasing the presence of boiling so that the texture of the material will be more lenient. (Numerical value is the higher). In this case the texture of the nuggets is also affected by moisture content. According to (Matz 1962) states that the higher the water content of a foodstuff, the texture is more soft and according to (priyawiwatkul et al. 1997) prodak texture of food affected its ability to bind water.

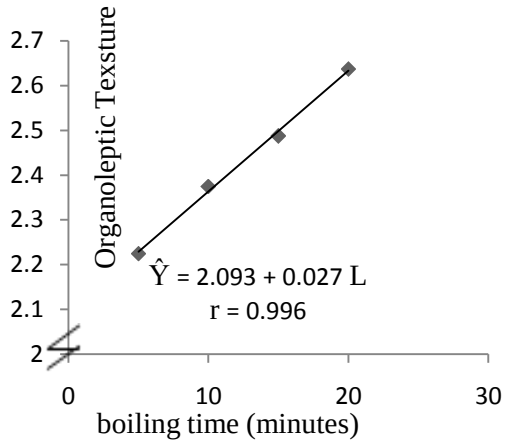


Fig. 6 Graph boiling time on organoleptic texture

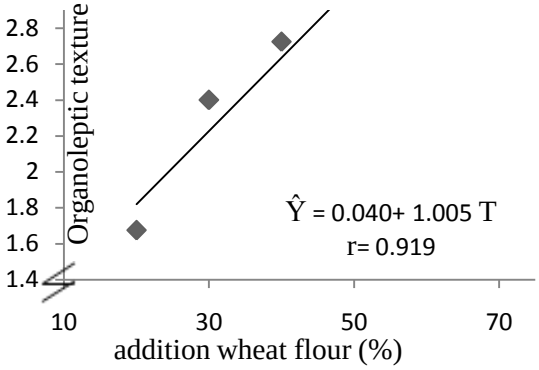


Fig. 7 Graph addition of wheat flour on organoleptic texture

Effect of Wheat Flour Addition on the Texture

From the analysis of variance attachment 4 can be seen that the addition of wheat flour provides highly significant effect (P <0.01) in the texture. Differences each - each level. That no significant T1 to T2, highly significant with T3 and T4. Value organoleptic texture highest in T4 treatment (50%) 2.925% and the lowest at T1 (20%) 1.675%. The higher the addition of wheat flour were added in the manufacture of rubber seed nuggets then occur and to more clearly seen in Fig. 7.

From Fig. 7It can be seen that the higher the addition of flour then texture increases. This is because flour can coat the

material at the nugget, the more flour then more chewy nuggets so panelist more like it. This is in accordance with the opinion (Amin, 2010) that the flour actually provides a good appearance at the nugget, and can coat and tie additional material in order to obtain nuggets that have a chewy texture.

Interaction Effect of Boiling time and Additions to the Appearance Texture Wheat Flour

From the list of variance Appendix 4 can be seen that the interaction between the boiling time and the addition of wheat flour had no significant effect (P> 0.05) to the value of organoleptic texture nugget rubber seed produced so LSR test was discontinued.

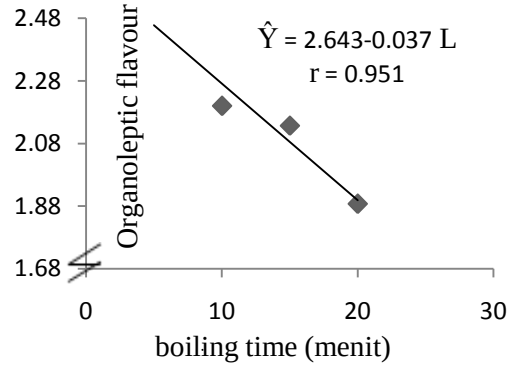


Fig. 8 Boiling old relationship with Seeds Rubber Appearance Aroma

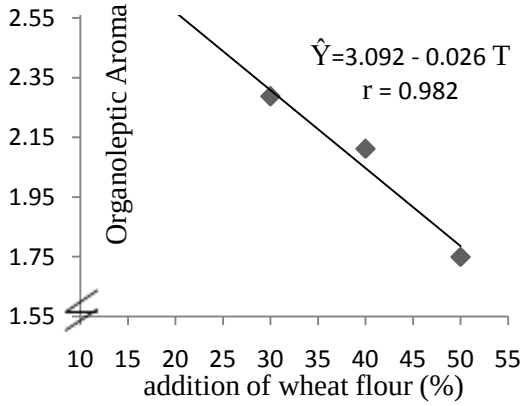


Fig. 9 Effect of wheat flour addition onaroma

*3.6. Organoleptic Aroma*

Effect of Boiling Old Rubber seeds to Aroma

From the analysis of variance attachment 5 can be seen that the boiling time provides highly significant effect (P <0.01) in the aroma nugget rubber seeds.

That no significant L1 to L2, highly significant with L3 and L4. Value organoleptic aromas highest in L1 treatment (5 minutes) as much as 2,488% and the lowest L4 (20 minutes) that is equal to 1,888% to more clearly seen in Fig. 8.

From Fig. 8 It can be seen that the longer boiling the organoleptic aroma decrease, This is because the boiling time causes damage to protein in the seeds of the rubber, the damage these proteins affect the scent produced so that the panelists do not like the scent nugget on appropriate treatment .This L4 with the statement Vaclavik et al (2008) protein denaturation is the process of changing the complete structure and characteristics of proteins from the disturbance of the interaction of secondary, tertiary, and quaternary structural such as temperature, due to thermal denaturation cause the molecules that make up proteins move very quickly. so that the hydrophobic nature of the protein to be open. As a result, the heat, the molecules will move faster and break the hydrogen bonds.

From the analysis of variance attachment 5 can be seen that the addition of wheat flour provides highly significant effect (P <0.01) in the aroma. Differences each - each.

No significant T1 to T2, highly significant with T3 and T4. Organoleptic aromas highest in T1 treatment (20%) 2.563% and the lowest was T4 (50%) 1.750%. The higher flour is added to the manufacturing of rubber seed nuggets then happened and for more details can be seen in Fig. 9.

The more the addition of wheat flour, the aroma caused to decrease. This is because the odor generated from the nugget comes from the mixture in the form of raw materials

such as condiments and eggs disappear as much flour is added. The higher the flour then diminishing the smell of the materials used in the manufacture of rubber seed nuggets. A panelist tinggkat then tended to decline. According to (Wirakartakusumah, 1997) nugget is determined by the content of water, fat and carbohydrates. The high water content will produce a scent that is missing or smell of the nugget will be reduced.

Interaction Effect of Boiling Old Flour And Additions to Test Appearance Aroma

From the list of variance Annex 5 can be seen that the interaction between the boiling time and the addition of wheat flour had no significant effect (P> 0.05) to the value of organoleptic aromas nugget rubber seed produced so LSR test was discontinued.

## Conclusion

The boiling time provides highly significant effect on protein content, ash content test 0rganoleptik texture and aroma, no significant effect on organoleptic taste.Addition of wheat flour provides highly significant effect on protein content, ash content, and organoleptic taste, texture, and aroma.

## References


[1] Deman JM,. 1997. *Kimia Makanan*. Edisike2.Penerjemah Padmawianata K, Bandung Terjemahandari :*Food Chemistry*: Penerbit ITB

[2] Haryanto,B., 1992. PotensidanPemanfaatanGandum. Kanisius, Yogyakarta.1992 Potensi dan Pemanfaatan Sagu. Yogyakarta: kanisius Press.

[3] Matz. S.A 1962. Water infood. The Avi publishing Company mc Westport, Connectieut

[4] RabahdanAbdalla, 2012. Decolorization of Acacia Seyal Gum Arabic.Annual Conference of Postgraduate Studies and Scientific Research Hall, Khartoum.Republic of Sudan.

[5] Sudarmadji, S., B. Haryono, danSuhardi. 1984. Proseduranalisauntukbahanmakanandanpertanian. Liberty. Yogyakarta

[6] Swaminathan. M. 1974. *Effect of Cooking and Heat Processing on TheNutritiveValue of Food*. Di dalam Essentials of food and nutrion.Ganesh and Company Madras. India. Vol 1. P. 384-387

[7] Vaclavik, Vickie. A dan Elizabeth W. Cristian. 2008. *Essential of Food Science Third Edition*. Springer Science + Business Media : New York

[8] Wirakartakusumah. 1997. Tinjauan aspek mutu dalam kegiatan industry pangan.

# Factors Affecting Farmers Decision to Convert Wetland

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## Abstract

This study aims to determine the rate of conversion of wetland area the last five years, and the factors that influence the decisions of farmers converting land. This research was conducted in Kecamatan Kualuh Hilir, Kabupaten Labuhanbatu Utara. Sampling method was used simple random sampling strata based on the large number. The analytical method was used descriptive, measurement scale, the least squares trend. Results of the study is that the rate of conversion of wetland in Sub KualuhHilir decreased by 7.36%. While wetland conversion rate based on primary data has decreased by 14.19% annually. In general it can be seen that 59.24% of respondents stated quite agree on the factors of land, agronomy, prices, income, climate, and environmental policies affecting farmers converting paddy fields.

Keywords: affecting farmer, wetland conversion, factors influence decision

## 1. Introduction

Land is a natural resource strategic development. Almost all sectors of the physical development needs land, such as agriculture, forestry, housing, industry, mining, and transportation. From the economic side, the land is still the main input of the production of a commodity. The amount of land used for the production activities in general is a derived demand resulting from commodity demand. Therefore, the development of land requirement for each production activity will be influenced by the growing demand of any commodity.[1]

In line with increased activity in commodity demand, the need for land is also increasing rapidly. While the availability and land essentially unchanged. Although the quality of land resources can be improved, the quantity in each region is relatively fixed. In these conditions, the increased need for land for production activity will reduce the availability of land for production activities. This causes frequent conflicts of interest and conversion.[2]

Land conversion is a change in the function of some or all of the land area of the original function (as planned) into another function which becomes the negative impact (the problem) to the environment and the potential of the land itself. Land conversion can also be interpreted as a change to other uses due to factors that broadly cover the need to meet the needs of a growing population numbers and increasing demands for a better quality of life.[3]

The growth followed a growing population with housing needs to make farmland is reduced in many areas. Land of the narrow increasingly fragmented due to the need for housing and industrial land. Farmers prefer to work in the informal sector of the last in the agricultural sector. The attractiveness of the agricultural sector continues to decline and farmers also tend to release their land ownership. The release of land ownership tends to be followed by changes in land use or often called the land conversion.[4]

Landowners do the conversion of cropland is mainly wetland to other uses therefore expect to benefit more. Economically, agricultural land, especially paddy, higher selling prices because it usually is in a thriving location. However, to peasants and farm workers, land conversion into a disaster because they could not change jobs. Farmers are getting stuck with increasingly limited opportunities for employment that will cause social problems that complicated.[5]

One of the areas that suffered the conversion of land in Sumatra Utara is KabupatenLabuhanBatu Utara. BPS data, in 2011 there is a decrease ear vast rice fields in KabupatenLabuhanBatu Utara from 35 771 ha to 31 375 ha in 2012. It appears that there is a decrease in the period of one year amounted to 4,396 hectares, it indicated the symptoms of wetland conversion in KabupatenLabuhanBatu Utara resulting in land area in the district will decrease wetland area in the future. Agricultural land is diminishing,especiallypaddyfieldsinKabupatenLabuhanBatu Utara, certainly will influence the production of paddy and rice production in the District. More extensive data wetland and paddy production in KabupatenLabuhanBatu Utara in five years is shown in Table 1.

Tablel 1. Land and Production of Rice

| Year | Wetland (Ha) | Production (Ton) | % Changes Land |
|---|---|---|---|
| 2008 | 59.491 | 258.787 | |
| 2009 | 32.376 | 152.523 | - 45,58 |
| 2010 | 40.815 | 189.871 | 26,06 |
| 2011 | 35.771 | 178.855 | -12,36 |
| 2012 | 31.375 | 155.250 | -12,29 |
| Average Change | | | -8,83 |

Source :KabupatenLabuhanBatu Utara in Figures Various Year of Publication

Tabel 2. Land Area, Production and Productivity of Rice in KecamatanKualuhHilir

| Year | Land Area (Ha) | Production (Ton) | Productivity (Ton/Ha) |
|---|---|---|---|
| 2000 | 26.250 | 116.442 | 4,43 |
| 2001 | 23.478 | 101.303 | 4,31 |
| 2002 | 22.561 | 96.671 | 4,28 |
| 2003 | 20.967 | 83.984 | 4,00 |
| 2004 | 19.731 | 81.236 | 4,11 |
| 2005 | 18.580 | 70.281 | 3,78 |
| 2006 | 17.580 | 80.235 | 4,56 |
| 2007 | 19.501 | 80.418 | 4,12 |
| 2008 | 17.064 | 78.238 | 4,58 |
| 2009 | 15.442 | 66.015 | 4,27 |
| 2010 | 15.442 | 63.023 | 4,08 |
| 2011 | 12.461 | 25.200 | 2,02 |
| 2012 | 13.256 | 77.290 | 5,83 |
| 2013 | 11.075 | 36.640,90 | 3,30 |

Source :KabupatenLabuhanBatu Utara in Figures Various Year of Publication

Kecamatan Kualuh Hilir is one of sub distric in KabupatenLabuhanbatu Utara in recent years continue to have the conversion of land, especially agricultural land. This resulted in the conversion of agricultural land in Kecamatan Kualuh Hilir especially paddy rice tends to decrease. Land most experienced conversion is a type of wetland into a dry land and non-agricultural land, such as is used for plantations and other things so forth. More data is the land area, production and productivity of paddy in KecamatanKualuhHilir in the last 14 years are shown in Table 2.

Based on the above problems, the study aims to determine the rate of conversion of wet land area, in addition, this study also aims to determine what factors are affecting farmers in the conversion of land in KabupatenLabuhanBatu Utara KecamatanKualuhHilir. The formulation of this research are:

1. How did the rate of conversion of paddy land in the last five years in the area of research?
2. What factors were affecting the farmer's decision to convert the land?

## 2. Literature

*2.1 Definition of wetlands*

Specifically land a development resource that has the characteristics of availability or extent of relatively fixed because the area changes as a result of natural processes (sedimentation). Moreover the suitability of land to accommodate community activities also tend to be specific because the land have different physical properties such as the types of rocks, minerals, topography and so forth.For farmers, the land has great significance because of their land to defend their lives with their families through farming and animal husbandry. Because the land is a factor of production in farming, then the tenure status of the land is very important with regard to the decision whether commodities to be commercialized.[6]

*2.2 Land Use*

Land use (land use) is any form of intervention (intervention) humans to land in order to meet their needs both material and spiritual. Land use can be grouped into two major groups, namely (1) the use of agricultural land, and (2) non-agricultural land useAgricultural land is the land designated for agricultural activities, such as rice fields, vegetable gardens, and others. Fields is a type of agricultural land use for management using the puddle. Therefore the fields is always a flat surface bounded by dikes to withstand waterlogging. Based on the type of irrigated rice fields are divided into three types: (1) technical irrigated fields, the form comes from the rice fields irrigation reservoir and flowed through the primary channel and further divided into secondary and tertiary canals through the building door divider; (2) rice field technical semi irrigation, the form of rice fields irrigation comes from reservoirs, but the government only controls tapper construction to regulate the importation of water, and (3) irrigated fields is simple, namely irrigation from springs and manufacture of the channel is made without a permanent building by the local community. As for the fact that in Indonesia there are rainfed, namely rice irrigation does not use irrigation. Watering in paddy fields based only on rain water.[7]

Wetland can be considered a public good, because in addition to the individual benefits for the owner, also provides benefits that are social. Paddy fields have functions that are widely associated with the benefits of direct, indirect benefits, and benefits default. Benefits directly related to, concerning the provision of food, provision of employment opportunities, providing a source of income for the people and the area, the cultivation of a sense of community facilities (gotongroyong), means the preservation of traditional culture, means of prevention urbanization and tourism facilities. The benefits are not directly related to its function as a means for conservationists. Benefits of default associated with its function as a means of education, and the means to maintain biological diversity.[8]

*2.3 Definition Conversion*

Land conversion is a change in the function of some or all of the land area of the original function (as planned) into another function which becomes the negative impact (the problem) to the environment and the potential of land. Land conversion can also be interpreted as a change to other uses due to factors that broadly cover the need to meet the needs of a growing population numbers and increasing demands for a better quality of life.[9]

*2.4 Factors Occurrence of Land Conversion*

Another factor that causes the conversion of agriculture is mainly determined by (1) the low value of the lease land (land rent); wetland located around the center of development compared to the rental value of land for housing and industry; (2) the lack of control functions and enforcement of the rules by the relevant institutions, and (3) growing prominence of short-term goal is to enlarge the local revenue (PAD) without considering sustainability (sustainability) natural resource decentralization

According to research conducted by Ilham (2003) in Irsalina (2010) is known to cause a conversion factor of external and internal sides of the farmer, the economic pressures during the economic crisis. This causes many farmers to sell their assets in the form of paddy fields to meet the needs of the impacted wetland increase conversions and improve land tenure to the parties of capital owners. Rainfed most experienced conversion (319,000 Ha) nationally. Paddy fields in Java with various types of irrigation experienced a conversion, respectively 310,000 ha of rainfed, irrigated fields Ha 234,000 technical, semi-technical irrigation rice field 194,000 ha and 167,000 ha irrigated rice simple.

Meanwhile outside Java conversion occurs only in irrigated and rainfed simple. The high conversion of irrigated rice in Java further strengthen the indication that control policies wetland conversion have not been effective. The occurrence of wetland conversion to oil palm trees for a variety of things, namely oil palm farm income is higher with lower risk, the sale value of collateral is higher gardens, palm oil farm production costs are lower and the limited availability of water.

2.5 Policy Aspects In Land Conversion

Various policy issues related to wetland conversion control has been a lot made. One aspect of the policy of wetland conversion stipulated in the Law of the Republic of Indonesia Number 41 of 2009 on the Protection of Agricultural Land Sustainable Food explained that what is meant by the Agricultural Land Sustainable Food is a farm plots is set to be protected and developed consistently to produce staple food for self-reliance, resilience, and national food sovereignty. While the protection of agricultural land sustainable food itself is defined as a system and a process to plan and establish, develop, utilize and develop, control and supervise food agricultural land and the region in a sustainable manner. The purpose of the protection of agricultural land sustainable food is (1) to protect agricultural land area and sustainable food; (2) ensure the availability of agricultural land sustainable food; (3) achieve independence, food security and sovereignty; (4) protect the ownership of agricultural land owned by farmers food; (5) increase the prosperity and welfare of farmers and the community; (6) improving the protection and empowerment of farmers; (7) increasing the employment of a decent life; (8) maintain the ecological balance, and (9) to realize the revitalization of agriculture. Agricultural land is protected only be converted for public use, which is regulated by government legislation. Conversion of land that has been set to do with the conditions as follows: (1) conducted a feasibility study strategic; (2) plans are exempt land conversion ownership rights of the owner, and the plot is provided in lieu of land converted[10].

Determination of perennial agricultural land is one policy option by some parties considered most appropriate to prevent the conversion of agricultural land. Basically perennial agricultural land is the establishment of the area as a conservation area, or protection, especially for agricultural businesses. Conversion of agricultural land to other uses prohibited by a statute legislation. If it can be effectively implemented then surely the conversion of land in conservation areas will not happen. Theoretically, the

assumption can be effected, this policy option is most effective to prevent the conversion of agricultural land.[11]

## 3. Material and Methods

This study used a case study (case study) the research done with a direct view of spaciousness, as the case study was a method that describes the type of research on a particular object for a certain period, or a phenomenon found in a place that is not necessarily the same as other areas[12].

### *3.1 Method of Determining Location*

The research area was determined by purposive in KecamatanKualuhHilir of LabuhanBatu Utara. This area is conversion of productive agricultural land, especially land.

### *3.2 Method of Sampling*

Determination of the sample of this research was to proportionate stratified random sampling that taking samples at random strata simple by the large number. The population in this study were farmers who convert agricultural land whether it is doing most conversions, and converting entirely.These farmers were members of the seven villageDesaKampung Masjid, Kuala Bangka, TelukBinjai, Sungai Sentang, Sungai Apung, TelukPiai And TelukMangedar. Based on information obtained from the head of the agricultural extension in Kecamatan Kualuh Hilir the number of the population that had wetland conversion in the amount of 1312 people.

If the population size is too large, then the sample size for descriptive studies do not need to be huge, just 5% alone is considered representative of the population. Determination of the number of samples for each village is as follows[13].

### *3.3 Data Collection Methods*

The data collected in this study consisted of secondary data and primary data.

Tabel 3. Number of Samples of Each Village

| Village | Population | Sampel |
|---|---|---|
| Kampung Mesjid | 183 | 9 |
| Kuala Bangka | 200 | 10 |
| Teluk Binjai | 143 | 7 |
| Sungai Sentang | 231 | 12 |
| Sungai Apung | 167 | 8 |
| Teluk Piai | 190 | 10 |
| Teluk Mangedar | 198 | 10 |
| Total | 1312 | 66 |

Source: Primary Data (Processed)

The primary data obtained from interviews directly to farmers as respondents using a questionnaire (questionnaire), which had been prepared in advance. While secondary data obtained from the agencies concerned.

### *3.4 Data Analysis Methods*

Formulation of the first problems were analyzed by using descriptive analysis by looking at the percentage changes in wetland area in KecamatanKualuhHilir within 5 (five) years.

According Sutandi (2009) in Astuti (2011), in the calculation of the rate of conversion of agricultural land use land depreciation equation. The rate of land conversion can be determined by calculating the rate of depreciation of land partially. The rate of land conversion may be determined by the difference between the total area of all t with the area prior to year-t (t-1). Then divided by the area year-to-t (t-1) are multiplied by 100 percent. This was done also in subsequent years in order to obtain the rate of conversion of land every year.The second problems were analyzed using descriptive rating scale measurements. Where respondents were given a few questions about

the factors that affect land conversion. Then the farmer respondents will be asked to declare the contents of agreement or disapproval of the inquiry into the four categories of the given numbers, namely (1) 4 agreed; (2), 3 quite agree; (3) 2 is less agreement, and (4) one does not agree. Then, if the farmer has declared kesetujuannyarespondent, then the figures tabulated the known prior to analysis. The analysis was based on figures obtained by the tabulation.

## 4. Result andDiscussion

*4.1 Wetland in KecamatanKualuhHilir*

The rate of conversion of paddy land in the KecamatanKualuhHilir within five years of extensive views of the percentage change in wetland per year can be seen in the following Table 4.

In Table 4 it can be seen that since the period of 2009-2013 decreased wetland area. Only in 2010 to agricultural land area has decreased from the previous year, in 2009 the land area equal to the land area in the year 2010 of 15,442 Ha. However, in 2011 the land area in KecamatanKualuhHiliragain decreased initially in 2010 amounted to 15,442 ha to 12 461 ha. Increased rice field area slightly occurred in 2012 amounted to 13 256 hectares and declined in 2013 to 11 075 ha. In Table 5 can be seen that the rate of wetland conversion.

The highest occurred in 2011 which amounted to 19.30% or occurpenunurunan rice field area of 2,981 Ha. The increase in rice field area which is equal to 795 hectares or 6.30% which occurred in 2012.

Overall, from 2009 to 2013 there has been a wetland conversion amounted to 7.36%. Thus wetland area in KecamatanKualuhHilir shrinkage land. While the rate of conversion of wet land in KecamatanKualuhHilir based on primary data in Table 5.

Table 4. Conversion Rate Wetland Five Years in KecamatanKualuhHilir

| Year | Land Area (Ha) | Changes Land Area (Ha) | % Changes Land Area |
|---|---|---|---|
| 2009 | 15.442 | | |
| 2010 | 15.442 | 0 | 0 |
| 2011 | 12.461 | -2.981 | -19,30 |
| 2012 | 13.256 | 795 | 6,30 |
| 2013 | 11.075 | -2.181 | -16,45 |
| Average Change | | | -7,36 |

Source: Secondary Data (Procesed).

In Table 5 above shows that the vast palm plantation in KecamatanKualuhHilir from 2010 to 2014 continued to increase in the amount of 4.42%. Increased land area from 2010 to 2011 amounted to 8.22%, from 2011 to 2012 amounted to 5.39%, whereas the increase in the land area from 2012 to 2013 amounted to 6.03% and from 2013 to 2014 experience increase in land area of 2.47%.The rate of conversion of paddy land area in KecamatanKualuhHilir from 2010 to 2014 continued to decline. Rice field area in 2010 has decreased by 16.09% in 2011, a decrease in wetland area also occurred in the year 2012 amounted to 14.07%, in 2013 amounted to 21.82% and in 2014 decreased by 18.95%. Overall, a wetland area in Kecamatan Kualuh Hilir shrinkage of 14.19% annually.

Decrease in wetland area in KecamatanKualuhHilir caused by land conversion. Farmers converting paddy fields to other crops such as oil palm trees as a result of wet land is capable of producing only once a year, because the rice fields in KecamatanKualuhHilir is rainfed areas which can only be planted at the end of the year or on a rainy day only. It is also the cause of farmers only earn income once a year, and the income received by the farmers are not able to meet the

needs for the next year. Therefore, farmers prefer to replace the rice crop fields to palm trees.

The decline in rice field area can not be separated from the differences in land rent. The allocation of land use wetland to use plantation crops is to provide economic surplus (land rent) higher. Hence the desire of farmers to earn more profits (allocation of land on higher land rent) of the land is the reason farmers replace paddy rice commodities.

Table 5. Conversion Rate Wetland Petani Respondents Five Years in KecamatanKualuhHilir

| Year | Sawit Land (Ha) | % Change | Paddy Land (Ha) | % Change |
|---|---|---|---|---|
| 2010 | 334,5 | 0 | 88,96 | 0 |
| 2011 | 362 | 8,22 | 74,64 | -16,09 |
| 2012 | 381,5 | 5,39 | 64,14 | -14,07 |
| 2013 | 404,5 | 6,03 | 50,14 | -21,82 |
| 2014 | 414,5 | 2,47 | 40,64 | -18,95 |
| Average Change | | 4,42 | | -14,19 |

Source: Primary Data (Processed)

Table 6. Description of Tabulation Conversion Factors Affecting Wetland Based on Land Indicators

| Commentary | Criteria | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | S | | CS | | KS | | TS | |
| | N | % | n | % | N | % | n | % |
| • Soil replacement plant is able to produce | 36 | 54,54 | 27 | 40,91 | 3 | 4,54 | 0 | 0 |
| • substitute crop land is more beneficial | 35 | 53,03 | 30 | 45,45 | 1 | 1,52 | 0 | 0 |
| • Quality of paddy fields increased | 12 | 18,18 | 10 | 15,15 | 19 | 28,79 | 25 | 37,88 |

Source : Primer Data (Processed)

Table 7. Description Tabulation Conversion Factors Affecting Wetland Based Agronomy Indicators

| **Commentary** | **Criteria** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **S** | | **CS** | | **KS** | | **TS** | |
| | **n** | **%** | **n** | **%** | **n** | **%** | **n** | **%** |
| • Rice is more susceptible to pests | 51 | 77,27 | 15 | 22,73 | 0 | 0 | 0 | 0 |
| • Rice more use of labor | 45 | 68,18 | 21 | 31,82 | 0 | 0 | 0 | 0 |
| • Ability to post-harvest handling | 13 | 19,70 | 22 | 33,33 | 21 | 31,82 | 10 | 15,15 |
| • Mechanical cultivation easier replacement | 38 | 57,58 | 28 | 42,42 | 0 | 0 | 0 | 0 |
| • Irrigation smooth | 0 | 0 | 0 | 0 | 39 | 59 | 27 | 41 |
| • When harvesting crops shorter replacement | 28 | 42,42 | 38 | 57,58 | 0 | 0 | 0 | 0 |

Source : Primary Data (Processed)

Table 8. Description of Tabulation Conversion Factors Affecting Wetland Based Price Indicator

| Commentary | Criteria | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | S | | CS | | KS | | TS | |
| | n | % | n | % | n | % | n | % |
| • High cost production | 37 | 56,06 | 29 | 43,94 | 0 | 0 | 0 | 0 |
| • Price substitute commodities higher | 41 | 62,12 | 24 | 36,36 | 1 | 1,52 | 0 | 0 |
| • Price cropland replacement collateral selling higher | 42 | 63,64 | 24 | 36,36 | 0 | 0 | 0 | 0 |

Source : Primary Data (Processed)

Table 9. Tabulation Description Size Conversion Factors Affecting Wetland Based Income Indicator

| Commentary | Criteria | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | S | | CS | | KS | | TS | |
| | n | % | n | % | n | % | n | % |
| • Farming Substitute crops could provide for the family | 33 | 50 | 33 | 50 | 0 | 0 | 0 | 0 |
| • Income Higher replacement crop farming | 34 | 51,51 | 32 | 48,49 | 0 | 0 | 0 | 0 |

Sumber : Primary Data (Processed)

However, in general, farmers inKecamatanKualuhHilir have done wetland conversion to oil palm trees still grow rice activity. Rice farming is a side activity in addition to the palm oil business. This is because farmers want to get the rice production at the end of the year, which is only used in rice production to meet the needs of family life.

*4.2 Factors Affecting Farmers Convert Land*

Factors that influence farmers to convert land can be seen by the indicator - an indicator that there is previously given to the farmers whose land conversion affecting land conversion in KecamatanKualuhHilir.

Based on Table 6 above it can be seen that the main reason farmers land conversion is 54.54% or 36 out of 66 people with reason substitute crop land is capable of producing as desired. Amounted to 53.03% or 35 people to do the conversion on the grounds substitute crop land is more beneficial. While 37.88% of the farmers land conversion for assessing the quality of wetland decline. Therefore the main reason farmers replace rice commodity with another commodity is a commodity due to replacement land has an exchange value that is greater than rice, so it is judged that commodity more profitable replacement. While the description of the factors that affect conversion tabulation extensive wetland based agronomic indicators can be seen in Table 7 below.

Table 7 shows that 77.27% or 51 out of 66 inhabitants agreed to convert the grounds of rice are more susceptible to pests and diseases. 68.18% or by 45 people expressed more rice using labor, 33.33% or 22 people claimed to do the conversion for the ability to post-harvest handling is not good. While other reasons farmers do a conversion that is equal to 57.58%, or about 38 people on the grounds of plant cultivation technique of replacement is easier than in rice, and 57.58%, or about 38 people expressed converting land for crop replacement time is shorter.

The above table shows that the reason farmers do a conversion is 56.06%, or 37

people on the grounds of high cost of rice production in compare replacement crops. 62.12% or 41 mental states to do the conversion on the grounds substitute commodity prices higher. While 63.64% or 42 out of 66 mental states to do the conversion on the grounds the sale price higher collateral substitute crops. Here are the factors that affect farmers' land conversion based on indicators of income.

It can be seen on the chart above that the reason farmers do a conversion of 50% or 33 souls agree with the reasons crop farming replacement could provide for the family. While 51.51% or 34 mental states to do the conversion by reason of higher farm income replacement. This suggests that rice farming in KecamatnKualuhHilircan not provide benefits for farmers.

Based on Table 10 shows amounted to 57.58% or 38 out of 66 mental states to convert land for reasons more supportive weather crop replacement. While 100% states the grounds that rice farming depending on the season.

Table 10. Tabulation Description Conversion Factors Affecting Wetland Based Climate Indicator

| Commentary | Criteria | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | S | | CS | | KS | | TS | |
| | n | % | n | % | n | % | n | % |
| • The weather replacement plant more support | 38 | 57,58 | 28 | 42,42 | 0 | 0 | 0 | 0 |
| • Farming depending on the season | 66 | 100 | 0 | 0 | 0 | 0 | 0 | 0 |

Source : Primary Data (Processed)

Table 11. Tabulation of Description Conversion Factors Affecting Wetland Based Indicators Government

| Commentary | Criteria | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | SB | | CB | | KB | | TB | |
| | n | % | n | % | n | % | n | % |
| • Government Policy | 0 | 0 | 0 | 0 | 22 | 33,33 | 44 | 66,67 |

Source: Primary Data (Processed)

Table 12. Tabulation of Description Conversion Factors Affecting Wetland Based Environmental

| Commentary | Criteria | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | S | | CS | | KS | | TS | |
| | n | % | n | % | n | % | n | % |
| • Influenced other farmers who convert | 33 | 50 | 20 | 30,30 | 3 | 4,55 | 10 | 15,15 |
| • means and good infrastructure | 0 | 0 | 0 | 0 | 29 | 43,94 | 37 | 56,06 |

Source: Primary Data (Processed)

In general, the fields in KecamatanKualuhHilir israinfed. The weather is often erratic make the product quality and production declined.

Farmers also for the rainfed areas are not capable of producing the appropriate or expected, because the rice crops could be planted at the end of the year only or on a rainy day only. Precisely paddy farmers only do once a year. It is also why farmers only earn income once a year, and the income received by the farmers is not considered to be sufficient for the next year. It is this which is one of the reasons to replace commodity farmers plant rice in addition to requiring time and labor Luangan larger than a replacement of commodities such as palm oil and so forth. While the factors that affect farmers perform conversions based on indicators of government policy can be seen in Table 11.

The above table shows that 66.67% or 44 out of 66 said they had reason to convert souls with government policies that are not good, while 33.33% said they had unfavorable government policies. This is because the government has not been optimal in giving counseling to farmers in KecamatanKualuhHilir. The provision of government subsidies granted to farmers is also considered to be uneven, certain circles are only partially subsidized. While the price of a given government subsidies has not been categorized as low. While the factors that affect farmers perform conversions based on environmental indicators can be seen in Table 12 below.

Based on Table 12 above can be shown that 50% or 33 mental states to do the conversion on the grounds affected other farmers who convert. Environmental factors also become a support farmers to convert land. One of them is to see the glory of farmers. Farmers who have already converted are considered to be sufficient for life and can set aside income for the purposes just in case and as an investment in the future. While 56.06% or 37 out of 66 souls meyatakan farmers do the conversion because infrastructure is not good.In general it can be seen that 59.24% of respondents stated quite agree on the factors of land, agronomy, prices, income, climate, and environmental policies affecting farmers converting paddy fields. The big difference in the value of the rice commodity with another commodity raises the desire of farmers to obtain surplus or gain more by converting land.

## Conclusion

Conclusion from the research that has been done, then people can obtain some conclusions regarding the issues examined in the field. Here is the conclusion.1. The rate of conversion of wet land in KecamatanKualuh from 2009 to 2013 by 7.36%. While the rate of conversion of wet land in KecamatanKualuhHilir based on primary data shrank by 14.19% annually.2. In general it can be seen that 59.24% of respondents stated quite agree on the factors of land, agronomy, prices, income, climate, and environmental policies affecting farmers converting paddy fields.

## References

[1] Hanafiah, K. A. (2013). *Dasar-dasarIlmu Tanah*. Jakarta: Rajawali Press.

[2] Sitorus, S. 1985. *EvaluasiSumberDaya Lahan*. PenerbitTarsito. Bandung.

[3] Ilham, M., Widiatmaka, S., Sarwono. 2003. *PerkembangandanFaktor-Faktor Yang MempengaruhiKonversiLahanSawah Serta DampakEkonominya*. IPB Press. Bogor.

[4] Tarigan, R. 2006. *Perencanaan Pembangunan Wilayah*. EdisiRevisi. BumiAksara. Jakarta.

[5] Jayadinata. 1997. *Tata Guna Tanah Dalam Perencanaan Pedesaan Pekotaan dan Wilayah*. ITB Press. Bandung.

[6] Hardjowigeno, S. (2011). *EvaluasiKesesuaianLahandanPerencanaanTatag unaLahan.* Cetakankedua. GadjahMada University Press. Yogyakarta.

[7] Nurhayati, H. 1986. *Dasar-dasarIlmu Tanah*. PenerbitUniversitas Lampung. Lampung.

[8] Irianto, G. (2002). *AlihFungsiLahan, Kekeringan, danKetahananPangan*. Jakarta. Papas SinarSinanti.

[9] Eddy, R danAbdulmutalib, T. (1998). *Pembangunan danKonversiLahan*. PenerbitUniversitas Lampung. Lampung.

[10] Arikunto, S. 2010. *ProsedurPenelitianSuatuPendekatanPraktek*. RinekaCipta. Jakarta.

[11] Mukhtar. 2013. *MetodePraktisPenelitianDeskriptifKualitatif*. Referensi GP Press Group. Jakarta.

[12] Sudirja, R. 2008. *MewujudkanKedaulatanPanganMelaluiKebijakan PengelolaanLahanPertanianPangan*. IPB Press. Bogor.

[13] Sugiyono. 2008. *Metode Penelitian Kuantitatif Kualitatif R & D*. Alfabeta. Bandung.

# The Occurrence of Somaclonal Variation on The Pineapple *In vitro* Culture as Detected by Molecular Markers

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## Abstract

Pineapple was the third of world important fruit, thus the research pertaining of this fruit had been conducted rapidly, including its *in vitro* aspect. The raising concern of *in vitro* methods was the occurrence of somaclonal variation on the plantlets. This research was conducted in order to determine the molecular profile of the pineapple plantlets derived from terminal shoots and crown explants (6 samples each) on the 4th sub culture (using MS media with 2.0 mg/ml BA). Five RAPD primers (OPA02, OPA03, OPA07, OPA13, and OPA19) were used to produce bands which were then scored manually, the Dice Sorensen coefficient similarity were calculated on the NTSYSpc 2.10x. The results suggested that either plantlets derived from terminal shoot or crown explants demonstrated genetic variation. The crown explantss plantlets showed highest (0.9697) and the lowest (0.6000) similarity coefficient. On the average, the similarity coefficient of crown explants plantlets (0.8647) were the highest among other plantlets, which was suggested that the crown explantss produce more genetically uniform plantlets than other explants. Furthermore, it was revealed that the use of low BA on the 4th sub culture of pineapple *in vitro* culture induced somaclonal variation. To produce *in vitro* clonal pineapple the frequency of subculture and concentration of BA used must be reduced.

Keywords: Pineapple, somaclonal variation, RAPD, explants type

## 1. Introduction

Pineapple is a third world's most important fruit and cultivated almost every tropical region. Since its high economical value, the research regarding to this fruit had been conducted intensely, including the *in vitro* and molecular aspects. In the present, the pineapple *in vitro* technique was also applied as tools in plant breeding.

Indonesian Tropical Fruit Research Institute has been conducted a pineapple breeding program which were produced many hybrids. However, these hybrids were still unreleased to the lack on an adequate seeds. The pineapple conservative propagation method only produced a limited number of seeds, thus the *in vitro* culture was one of the alternatives.

The first accomplishment pineapple *in vitro* culture was reported in 1978 [1] who used varied explants (crown, slip, and syncarp) to induce multiple shoots. Although Wakasa [2] successfully planted the plantlets to the field, he found some variation in leaves and spine morphology. Since then, the pineapple *in vitro* studies was expanded even some reported a production of thousand of shoots from single explants [3], [4], [5]-[7]. Separately, some research on the pineapple molecular aspects were conducted to detect the somaclonal variation on the *in vitro* plantlets using isozyme and RAPD (Rapid Amplified DNA Polymorphism) markers [8],

[9], [10]. Other factors that might induce somaclonal variation on *in vitro* plantlets were type of explants, frequency of subculture, and the use of plant growth hormone [11].

The unpublished research had reported somaclonal variation in all pineapple plants derived from *in vitro* propagation. Thus, the detection of somaclonal variation on the plant early stage had to be conducted to prevent more loss by the using the inappropriate seeds. The research was done in order to examine the genetic profiling of the 4th sub culture of pineapple *in vitro* plantlets.

## 2. Material and Methods

Plantlets of *in vitro* pineapple hybrids 18X5(10) derived from four different explants, namely hapas, crown which were grew on MS media [12] modified with 2 mg/ml BA. The 4th sub culture plantlets were chosen as the lowest sub culture frequency which the plantlets were usually brought to the acclimatization process.

DNA from 6 plantlets each from the same explants was extracted using CTAB method [13]. The extract DNA were then used as a template on RAPD reaction with 5 primers as recommended by [10] (Table 1). RAPD PCR reaction was performed with Taq PCR reaction mixture 4.25 µl (KAPPA) with 1 µl of RAPD, 1 µl (10 ng) of sample DNA, and $ddH_2O$ to a final volume of 12.5 µl reaction. PCR was performed by 45 cycles with the following programs: pre denaturation 95°C for 3 minutes, denaturation 95°C for 15 seconds, annealing 36 °C for 15 seconds, extension 72°C for 5 seconds, and final extension: 72°C for 10 minutes.

DNA amplification product was separated by electrophoresis at 50 V for 20 minutes. The band patterns were scored manually and automated using software BioDocAnalyzer. The Dice-Sorensen coefficient of similarity was conducted using the NTSYSpc 2.10x software [14].

Table 1. Primers used to detect somaclonal variation on pineapple *in vitro* plantlets

| Primers | Sekuens (5'-3') |
|---|---|
| OPA02 | TGCCGAGCTG |
| OPA03 | AGTCAGCCAC |
| OPA07 | GAAACGGGTG |
| OPA13 | CAGCACCCAC |
| OPA17 | GACCGCTTGT |

## 3. Result and Discussion

Somaclonal variation has become a major in pineapple micropropagation, thus early detection method to determine the plantlets genetic variability had been developed, included RAPD-PCR [15]. The RAPD reaction on the pineapple plantlets derived from four type of explants, namely hapas, crown, suckers, and aerial suckers were conducted using five primers. The DNA samples were extracted from each planlets that were originated from the same explants source. The gel images of those amplifications were presented in Figure 1 and 2.

Overall it was demonstrated that primers used in this study produced 3-10 bands, included at some monomorphic and at least one polymorphic bands. Primes OPA2 and OPA3 seems to produce the least polymorphic band on hapas and crown derived plantlets, whereas primer OPA07 produce the most polymorphic band on suckers and aerial suckers derived plantlets. All primers used were able to revealed genetic variability among the plantlets. The Dice-Sorensen similarity coefficient were calculated for each type of plantlets which was resulted that none of the explants produced 100% genetically uniform plantlets (none 1.000 similarity coefficient found-Table 2, 3, 4, and 5).

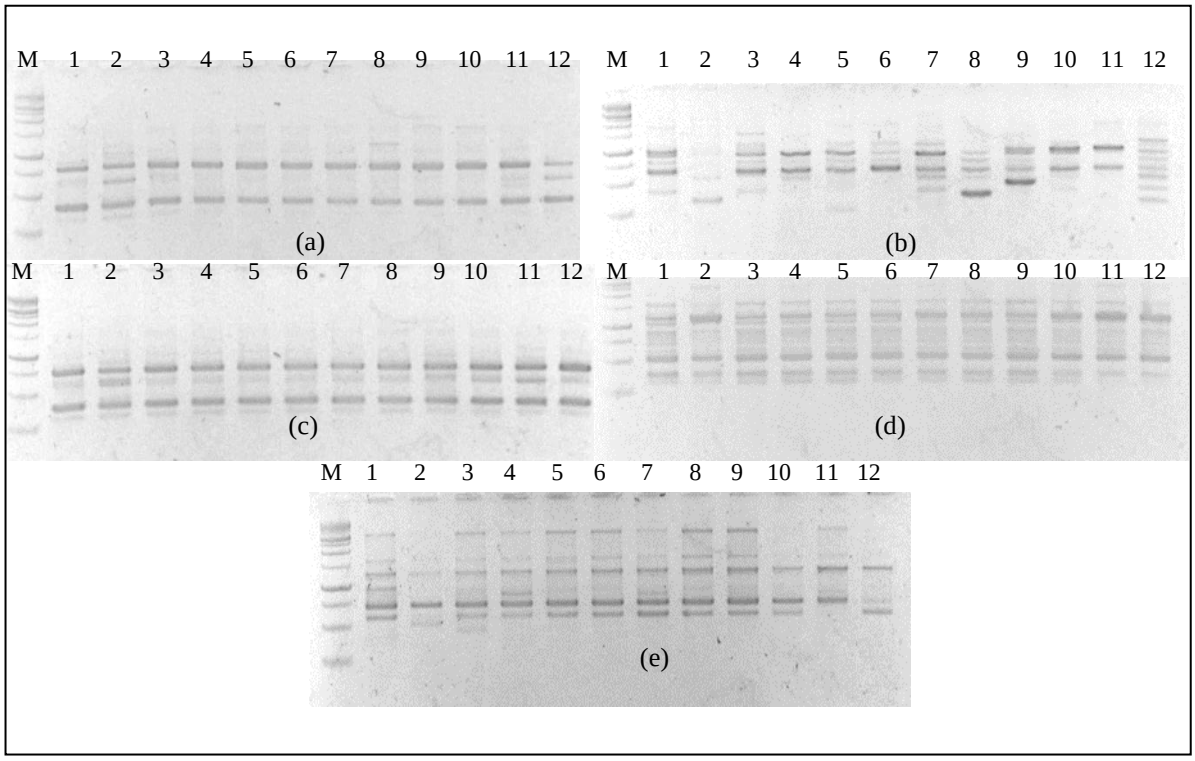


Figure 1. Gel Electrophoresis of RAPD analysis of 4th subculture pineapple in vitro culture, with primers: (a) OPA02, (b) OPA03, (c) OPA07, (d) OPA13, and (e) OPA17. (M: 100 kb marker, 1-6: plantlets derived from hapas explants, 7-12: plantlets derived from crown explants).

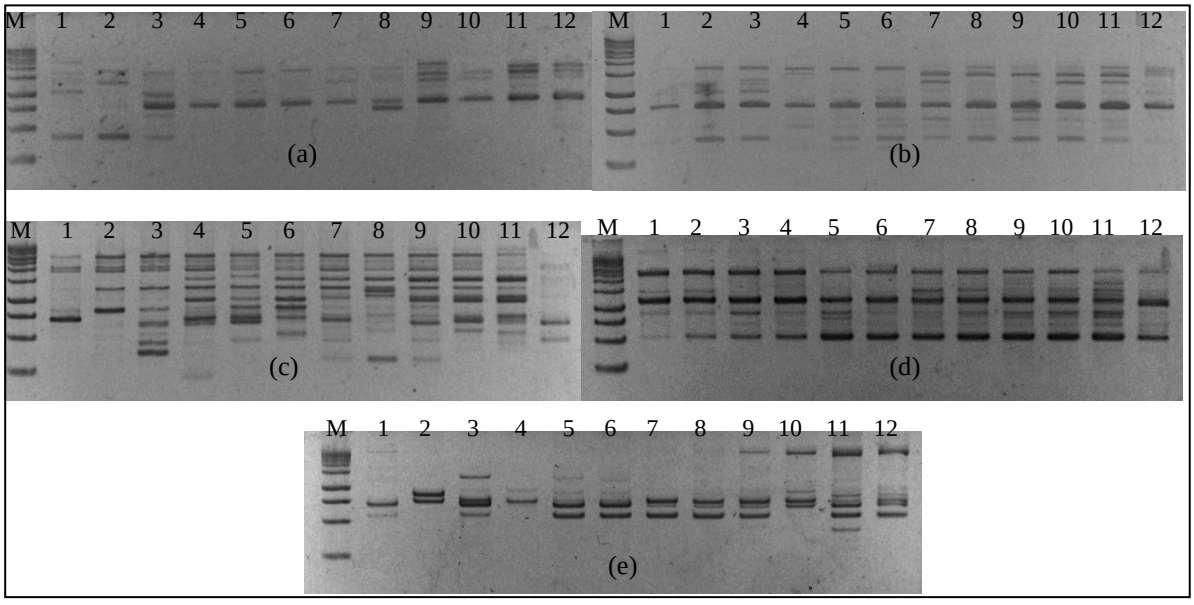


Figure 2. Gel Electrophoresis of RAPD analysis of 4th subculture pineapple *in vitro* culture, with primers: (a) OPA02, (b) OPA03, (c) OPA07, (d) OPA13, and (e) OPA17. (M: 100 kb marker, 1-6: plantlets derived from suckers explants, 7-12: plantlets derived from aerial suckers explants).

Although, this study did not involve the mother (explants source) plant, the result had already detected genetic variability among the explants that came from the same explants source which also confirmed by [15]. The earlier study also explained that the genetic variability further will result a somaclonal variation on pineapple. The genetic and epigenetic changes in plant *in vitro* culture induced somaclonal variation that might be occurred on far beyond rate that expected in nature [16]. Those changes included point mutation, transposition activity of mobile genetic elements, chromosomal rearrangement, or ploidy level changes which induced genetic instability [17].

Moreover, it was also revealed the hapas explants derived plantlets showed both the biggest (0.9697) and lowest value (0.6000). The average coefficient similarity from hapas, crown, suckers, and aerial suckers derived plantlets were 0.8177, 0.8647, 0.7263, and 0.7892, respectively.

Table 2. Dice-Sorensen coefficient similarity matrix of 4th subculture hapas explants derived pineapple plantlets

| S | H1 | H2 | H3 | H4 | H5 |
|---|---|---|---|---|---|
| H2 | 0.5806 | | | | |
| H3 | 0.8824 | 0.6207 | | | |
| H4 | 0.8824 | 0.6207 | 0.9375 | | |
| H5 | 0.9143 | 0.6000 | 0.9697 | 0.9091 | |
| H6 | 0.8824 | 0.6207 | 0.9375 | 0.9375 | 0.9697 |

*Note: S, Sample*

Table 3. Dice-Sorensen coefficient similarity matrix of 4th subculture crown explants derived pineapple plantlets

| S | C1 | C2 | C3 | C4 | C5 |
|---|---|---|---|---|---|
| C2 | 0.9474 | | | | |
| C3 | 0.9444 | 0.9474 | | | |
| C4 | 0.8750 | 0.8235 | 0.8750 | | |
| C5 | 0.8485 | 0.8571 | 0.9091 | 0.8966 | |
| C6 | 0.8421 | 0.8500 | 0.7895 | 0.7647 | 0.800 |

*Note: S, Sample*

Table 4. Dice-Sorensen coefficient similarity matrix of 4th subculture suckers explants derived pineapple plantlets

| S | S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|---|
| S2 | 0.6250 | | | | |
| S3 | 0.6667 | 0.8089 | | | |
| S4 | 0.7143 | 0.7059 | 0.6316 | | |
| S5 | 0.6667 | 0.6667 | 0.7907 | 0.8000 | |
| S6 | 0.6250 | 0.6842 | 0.7619 | 0.8235 | 0.9231 |

*Note: S, Sample*

Table 5. Dice-Sorensen coefficient similarity matrix of 4th subculture aerial suckers explants derived pineapple plantlets

| | AS1 | AS2 | AS3 | AS4 | AS5 |
|---|---|---|---|---|---|
| AS2 | 0.8571 | | | | |
| AS3 | 0.8571 | 0.9412 | | | |
| AS4 | 0.8108 | 0.7222 | 0.7778 | | |
| AS5 | 0.8293 | 0.8000 | 0.8000 | 0.9048 | |
| AS6 | 0.6667 | 0.6875 | 0.6875 | 0.7059 | 0.7895 |

Those values indicated that the crown explants produced more genetically similar plantlets compare to the other explants types as also indicated by [1]. It was reported that the genetic fidelity of *in vitro* plants were largely depends on explants source, the explants tissue can affect the frequency and nature of somaclonal variation [18].

The media used in this study was modified MS media which supplemented with 2 mg/ml BAP. In previous research, the application of commom cytokines such as BAP produced many (up to 31 shoots per explants) *in vitro* shoots [12]. However, it seems that the low level of plant hormone had already induced cytological changes in pineapple. It was had explained that hormonal element of culture medium are powerful agent of variation [18]. Furthermore, stress condition during *in vitro* culture, such as wounding, exposure to plant hormone and other specific elements on *in vitro* medium caused cytological changes [17].

The plantlets examined on this study came from the fourth subculture cycle. Usually, *in vitro* culture of pineapple was repeatedly conducted twice or more to induce more shoots. Reference [5] produced total of more than 120,000 shoots per explants within four time subculture and 75 days incubation period. In the present study, the four time subculture seem had already caused variation. The rapid multiplication of culture may affect genetic stability and thus lead to somaclonal variation [18].

## Conclusion

The application of 2 mg/ml BA on MS media and four time sub culture on the same media to induce pineapple *in vitro* multiple shoots might caused genetic variation of the plantlets which were grew from the same explants. Crown explants induced lower frequency of genetic variation than hapas, suckers, and aerial suckers explants. Therefore, the use of lower concentration of BA and less sub culture frequency were suggested in order to produce more genetically similar pineapple plantlets.

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## References

[1] K. Wakasa, Y. Koga, and M.Kudo, "Differentiation from in vitro culture of *Ananas comosus,"* Japan J.Breed 28(2): 113-121, 1978.

[2] K. Wakasa, "Variation in the plants differentiated from the tissue culture of pineapple," Japan J Breed 29 (1): 13-22, 1979.

[3] E. Kiss, J.Kiss, G.Gyulai, and L.E. Haszky, "A novel method for rapid micropropagation of pineapple," Hort. Sci. 30(1): 127-129, 1995.

[4] L.V. Be and P.C. Debergh, "Potential low-cost micropropagation of pineapple *(Ananas comosus),"* South African J. Bot. 72: 191-194, 2006.

[5] A.M. Hamad and R. M. Taha, "Effect of sequential on in vitro proliferation capacity and shoot formations pattern of pineapple (*Ananas comosus* L. Merr.) over different incubation periods," Sci. Hortic. 117: 329-334, 2008.

[6] A.M. Al-Saif A.B.M.S. Hossain, and R.M. Taha, "Effect of benzylaminopurine and naphthalene acetic acid on proliferation and shoot growth of pineapple *(Ananas comosus* L. Merr.*) in vitro,"* African J. Biotec. 10(27): 5291-5295, 2011.

[7] F. Farahani, "Micropropagation and growth of in vitro pineapple *(Ananas comosus* L. Merr.) in Iran," Plant Archives 14(1): 337-341, 2014.

[8] S. Feuser, K. Meler, M. Daquinta, M.P. Guerra, and R.O. Nodari, "Genetic fidelity of micropropagated pineapple (*Ananas comosus*) plantlets assessed by isozyme and RAPD markers*,"* Plant Cell Org.Tiss.Cult. 72: 221-227, 2003.

[9] M.D.M. Santos, G.C.S. Buso, and A.C. Torres, "Evaluation of genetic variability in micropropagated propagules of ornamental pineapple [*Ananas comosus* var. bracteatus (Lindley) Coppens and Leal] using RAPD markers," Genet. Mol. Res 7.4: 1097-1105, 2008.

[10] I. Roostika, N. Khumaida, and S.W. Ardie, "RAPD analysis to detect somaclonal variation of pineapple in vitro culture during micropropagation*,"* Biotropia 22(2): 109-119, 2015.

[11] M.W. Bairu, A.O. Aremu, and J.Van Stadden. 2011. Somaclonal variation in plants: causes and detection methods," Plant Growth Regul. 63: 147-173, 2011.

[12] T. Murashige and F. Skoog, "A revised medium for rapid growth and bioassays for tobacco tissue cultures," Physiologia Plantarum 15: 473-497, 1962.

[13] J.J. Doyle and J.L. Doyle, "A rapid DNA isolation procedure for small quantities of fresh leaf tissue," Phytochem Bull 19:11-15, 1987.

[14] F.J. Rohlf, NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 2.1.Applied Biostatics, New York, 2004.

[15] I. Roostika," Pengembangan metode organogenesis dan embryogenesis somatic pada nanas (*Ananas comosus* L. (Merr.) serta deteksi dini untuk mereduksi keragaman somaklonal. Doctoral Dissertation, IPB, Bogor: X + 141 hlm, 2012.

[16] Q.M. Wang and L. Wang, "An evolutionary view of plant tissue culture: somaclonal variation and selection," Plant Cell Rep 31: 1535-1547, 2012.

[17] A.S. Azman, C. Mhiri, M.A. Grandbastein, and S.M. Tan, "Transposable elements and the detection

of somaclonal variation in plant tissue culture: A review," Malays. Appl. Biol. 43(1): 1-12, 2014.

[18] A.R. Leva, R. Petrucelli, and L.M.R. Rinaldi, "Somaclonal variation in tissue culture: A case study with olive, In: Recent advance in plant in vitro culture, Intech, 123-150 pp, 2012.

[19] A.R. Zuraida, Shahnadz N.A.H., Harteeni, A., Roowi, S., CheRadziah, C.M.Z., and Sreeramanan S, "A novel approach for rapid micropropagation of maspine pineapple *(Ananas comosus L.)* shoots using liquid shake culture system," African Journal of Biotechnology Vol. 10(19): 3859-3866, 2011.

# Competitiveness of *Fusarium oxysporum*f.sp. c*ubense* VCGs 01213/16 (Tropical race 4) Among Several VCGs in Race 4 on Ambon Hijau Cultivar

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## Abstract

Competition is the general antagonism mechanism of microorganism. *Fusariumoxysporum*f.sp. *cubense* (*Foc*) is a deadlypathogenic fungi on banana cultivars. This pathogen had several Vegetative Compatibility Group (VCG) composing the different level of virulent on plant.The objectives of the research were to know the competitiveness of *Foc* isolate VCGs01213/16 among several VCG on Ambon hijau. The research was conducted at plant protection laboratory and screen house of Indonesian Tropical Fruit Research Institute Solok, from August 2012 to Feb 2015. The experiment was arranged in a randomized complete block (RBD) with seven treatments and three replications, each treatment consists of 6 plants. The treatments were A=VCG 01213/16, B=VCG 0120,C=VCG 0121, D=VCG 01219, E=VCG 01213/16+0120, F=VCG 01213/16+0121 and G=VCG 01213/16+01219. Parameter was observed is percentage of wilted plant, incubation period, disease severity index on leaves and corm and VCG analysis. The resultsshowed that *Foc* isolates VCG 01213/16 is the most competitive strains among other VCG in race 4. Result of VCGs analysis revealed that a VCG isolates were detected 100% from co-inoculation it with other VCG. Single or co-inoculation of *Foc* isolates VCG 01213/16 caused 69,6 to 94,8% plant wilted. Co-inoculation of *Foc* isolates VCG 01213/16 with VCG 0120 delayed the incubation period of disease until 10 days and decreased the percentage of wilted plant until 26.6%. Co-inoculation of*Foc* isolates VCG 01213/16 with VCG 0120 reduced the disease severity on leaves and corm and significant different with at single inoculation of *Foc* VCG 01213/16.

Keywords: Banana, Fusarium oxysporumf.sp. cubense, Vegetative Compatibility Group, competition, dominance

## 1. Introduction

Competition is one of three general antagonism mechanism on direct microbial. Competition occured in soil for nutrient and for infection sites on in root tissue. *Fusarium oxysporum* is well studied among the rizosphere microflora and representing a competition model of microorganism [1].

Some studies have observed that the competition process occurs between non pathogenic or pathogenic strain of *Fusarium oxysporum*[2]. *Fusarium* spp. was competing for root colonization and might directly or indirectly inhibit their metabolic activity [3]. Strains have able to colonize in a site and one strain hampered colonisation of the other strain [4].

*Fusarium oxysporum* Schlecht f. sp. *cubense* (*Foc*) (E.F. Smith) Snyder & Hansen belongs to the pathogenic strain of *Fusarium*

*oxysporum*, as a causal agent of the most catastrophic Fusarium wilt disease on banana. This soil borne pathogen has paralyzed million hectares banana plantation all over the world [5.6.7.8.9.10]. First occurrence was in Tropical America (Costa Rica and Panama) affecting banana "Gros Michel" in 1890, therefore known as the Panama disease[11]. Panama disease has been a major disease also in Australia, Asia, Central and South Africa [11.12.13.14.15]. In Indonesia, *Fusarium* wilt has ruined thousands hectares of commercial and traditional plantations [16.17] from Nanggroe Aceh Darussalam to Papua Provinces [8.9]. As well as other forma specialist, *Foc*are grouped into several groups. Recently, vegetative compatibility groups (VCGs) is an applicable method compared to subdivide based on previous classification namely race group. The VCG is subdivide the population in group according the knowledge of particular interest in asexual fungi that can exchange genetic information via heterokaryotic [18.19]. Until now, 21 VCG of *Foc* had been found in the world, 15 of VCG are in Asia [20.21]. 10 isolates of *Foc* VCG derived from the 16 provinces in Indonesia [8]. Pathogenicity level of VCG *Foc*to several banana cultivars are well documented. It has been reported that 10 VCG of *Foc*have different level of virulence against 8 banana cultivars [9]. *Foc* VCG 01213/16 (Tropical race 4/TR4) is known as the most dominant and virulent strain. The domination of VCG 01213/16 in the fields may be caused by this VCG is more competitive than other strains[21.26]. However there was no accurate data to support it, whether the other VCG also existed in the rizosphere of banana plant. On the other hand it was confirmed that competititivess ability of the non pathogenic to pathogenic *Fusarium oxysporum* occured on tomato plants can decrease plant wilt [3]. Based on no report regarding to the existence, competitiveness and dominance of the strains of *Foc* in a plant. Therefore, the objectives of this experiment were to know the competitiveness of VCGs 01213/16 of *Foc* among several VCGs in race 4 groups on banana cultivar Ambon hijau.

## 2. Material and Methods

The research was done at Plant Protection laboratory and green house of Indonesian Tropical Fruit Research Institute. Pathogenicity test has been conducted from August 2012 and the VCG test was confirmed up to February 2015.

**Greenhouse experiment.** To compare the aggressiveness of the single and combination of VCG *Foc*, four VCG isolates were used in green house assay. The experiment was arranged in completely randomized block design with six replications.Each replication consisted of a single plant per isolates or combination. The treatments tested were: A=VCG 01213/16, B=VCG 0120,C=VCG 0121, D=VCG 01219, E=VCG 01213/16+0120, F=VCG 01213/16+0121 and G=VCG 01213/16+01219.Soil temperature in the greenhouse experiment was at 30-35$^{o}$C in range.

The *Foc* VCG which be tested were *Foc* collection of Indonesian Tropical Fruit Research Institute. Each isolate was grown on PDA for 7-10 days at room temperature (25 ± 2°C) under 24 h of fluorescent lighting. Spore suspensions for inoculation were prepared by flooding cultures with ≈50 ml of sterile distilled water (SDW), dislodging conidia with a disposable hockey stick, filtering through sterile cheese cloth to avoid agar residues, and adding water to obtain a final volume of 100 ml. Spores were enumerated using a hemocytometer, and the spore concentration was adjusted to $10^{6}$ conidia ml–1 by adding Sterile Distilled Water (SDW). SDW was used as a control.

The cultivar was tested is Ambon hijau cultivar (Cavendish subgroups) from tissue culture propagation with up to 5-6 leaves. Prior inoculation, root tips of the evaluated plants were cut 1 cm using sterile scissor.

Inoculation was done by dipping root technique [22]. Each wounded root tips plants was dipped for 15 minutes in suspension of *Foc* conidia. Un-inoculated plants were used as control. Subsequently, inoculated and inoculated plants were planted to double cup plastic 250 ml contained nutrient solution (hyponex[tm]) (outer cup) and sterile sand medium (inner cup).

**Data analysis.** a) Incubation period: a time from time of inoculation to the first appearance of symptom, b) disease incidence: was observed at the end of observation using the following formulae:

$$P = \left[\frac{T_1}{T_2}\right] \times 100\%$$

P=disease incidence, T1=number of infected plants and $T_2$=number of observe plants

c) leaf disease severity index (LDSI): was assessed using scoring system as follow [23]: 1=No streaking or yellowing of leaves, plant appears healthy; 2=Slight streaking and/or yellowing of lower leaves; 3=Streaking and/or yellowing of most of the lower leaves; 4=Extensive streaking and/or yellowing on most or all of the leaves; 5=Dead plant, d) corm disease severity index (CDSI: was assessed at 2 month after inoculation (the end of experiment)destructively). Prior the observation, corm was cleaned from roots and soil, and cross sectioned at the middle part. Observation was done using [24] scoring system as follow: 1=Corm completely clean no vascular discoloration; 2=Initial points of discoloration in vascular tissue; 3=Discoloration of up to 1/3 of vascular tissue; 4=Discoloration between 1/3 and 2/3 of vascular tissue; 5=Discoloration greater than 2/3 of vascular tissue; and 6=Total discoloration of vascular tissue.

Index of disease severity on leaves and corm are calculated by the formula:

$$I = \Sigma \frac{\text{scale value x number of plants of each of the scales}}{\text{Number of plants}}$$

Data were analyzed by variance, if the results obtained are significantly different, further tested for Least Small Differences (LSD) on the 5% level.

**Vegetative compatibility tests.** This test was to confirm the aggressiveness and dominance of a VCGs in vitro. *Foc* samples were taken from 1 to 3 leaves of tested plant showing external specific symptom (Figure 1). The leaf were cultured on 1/3 potato dextrose agar (PDA) supplemented with 50 ppm streptomycin. The culture was incubated at room temperature for 2-3 days. Subsequently, isolates were purified by performing single spore technique (sst) as described by [23]. Purified *Foc* isolates were then analyzed by Vegetative Compatibility Group test. *Foc*isolates and the VCGs testers of *Foc*was determinedby complementation (heterocaryon) tests on MM using *nit* mutants. When a *nit* mutant formed wild-type heterokaryotic growth at the contact zone with testers VCG 01213/16 or 0121 and 01219; their parent isolate was regarded as similar VCG.

## 3. Result and Discussion

The result of pathogenicity test carried out on Ambon hijau cultivar against 4 VCGs isolates of *Foc* are presented in Table 1. The examined *Foc* isolates had compatible to Ambon hijau cultivar. Most of tested plant produced typical symptom of wilting and yellowing leaveson plant. Ambon hijau cultivar is a susceptible cultivar to *Foc* race 4 [27].

The virulence of *Foc* isolates can be determined from percentage of host wilting. The result has shown that the percentage of wilted plant is varies, it ranges from 26,71 to 94,84%. The highest percentage of wilted plant was found on single inoculation of *Foc* isolate VCG 01213/16 on tested plant, otherwise the lowest is on plant was inoculated with *Foc* isolate VCG 01219. VCG 01213/16 is known as a highly virulent strain

[22]. Beside the *Foc* isolates having a different level of virulence, the difference in percentage of plant wilted may occur due to the plant exhibit a difference resistance mechanism against each *Foc* isolate. [26] pointed that there are variation in virulence level among each VCG isolate in a group of cultivar. Plant has a specific defense mechanism against the pathogen and thus built a different response to deal with the pathogen [27].

Furthermore, co-inoculated *Foc* isolates VCG 01213/16 with other VCG included in race 4 affected the percentage of plant wilt. If *Foc* isolates VCG 01213/16 and VCG 0121 or VCG 01219 co-infected in root tissue, even no significant different on its value, percentage of wilt is lower relatively than on single inoculation of *Foc* isolates VCG 01213/16.

Table 1. Mean of percentage of wilt of banana cultivar Ambon hijau was caused by isolates of *Foc* VCG 01213/16, VCG 0121, VCG 0120 and VCG 01219 in single and combination at 10weeks after inoculation (wai)

| Treatment | Percentage of wilt (%) * |
|---|---|
| VCG 01213/16 | 94,841[a] |
| VCG 0120 | 33,586[c] |
| VCG 0121 | 87,158[ab] |
| VCG 01219 | 26,71[c] |
| VCG 01213/16 + 0120 | 69,578[b] |
| VCG 01213/16 + 0121 | 85,141[ab] |
| VCG 01213/16 + 01219 | 72,1[ab] |
| CV | 34,53 |

*Means followed by the same capital letters within the same row and followed by the same small letters within the same coloums are not significantly different at 5% level of LSD.*

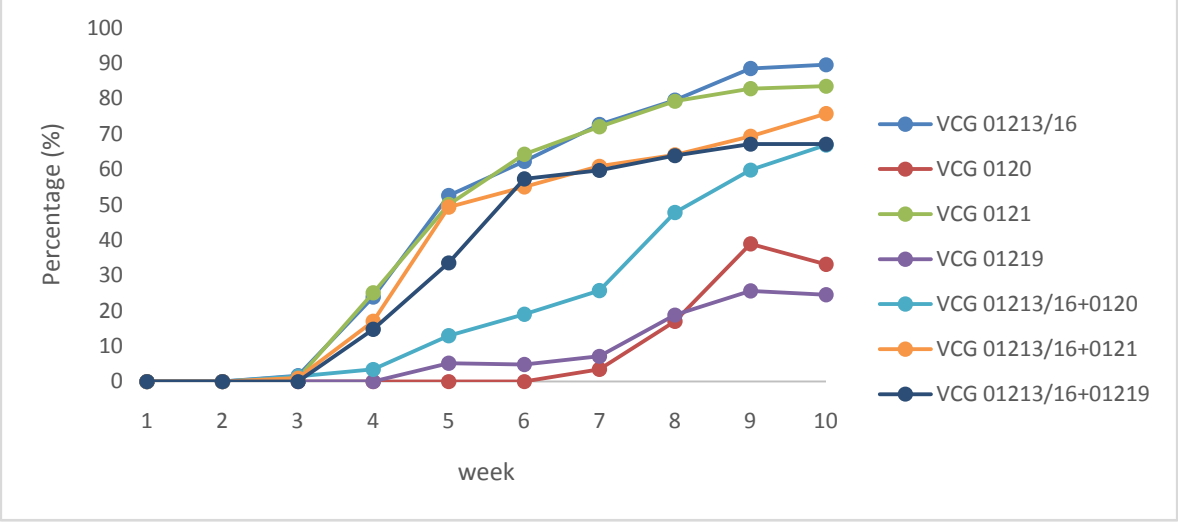

Figure 1. Progression of wilted of Ambon hijau caused by single or co-inoculation of several VCG *Foc* isolates.

Table 2. Mean of incubation period of *Foc* VCG 01213/16, VCG 0121, VCG 0120 and VCG 01219 and percentage of its VCG appearance in single and co-inoculation at 10wai.

| Treatment | Incubation period (day) ** | % of VCG appearance | | | |
|---|---|---|---|---|---|
| | | 01213/16 | 0120 | 0121 | 01219 |
| VCG 01213/16 | 24,667[c] | 100 | | | |
| VCG 0120 | 46,833[a] | | 100 | | |
| VCG 0121 | 23,444[c] | | | 100 | |
| VCG 01219 | 45,611[a] | | | | 100 |
| VCG 01213/16 + 0120 | 34,667[b] | 100 | 0 | | |
| VCG 01213/16 + 0121 | 27,667[c] | 100 | | 0 | |
| VCG 01213/16 + 01219 | 29,00[bc] | 100 | | | 0 |
| CV | 25,43 | | | | |

*Means followed by the same letters within the same row are not significantly different at 5% level of LSD.*
***) mean of incubation period were calculated from number of wilted plant.*

Table 3. Disease severity index on leaves and corm of banana Ambon hijau infected by *Foc* VCG 01213/16, VCG 0121, VCG 0120 and VCG 01219 by single and co-inoculation, 10 wai

| Treatment | Disease severity of | |
|---|---|---|
| | Leaves | Corm |
| VCG 01213/16 | 4,78[a] | 6[a] |
| VCG 0120 | 2,67[c] | 1,27[c] |
| VCG 0121 | 4,61[ab] | 5,89[ab] |
| VCG 01219 | 2,33[c] | 1,27[c] |
| VCG 01213/16+0120 | 4,0 [ab] | 4,94[b] |
| VCG 01213/16+0121 | 4,45[ab] | 5,44[ab] |
| VCG 01213/16+01219 | 3,94[b] | 5,44[ab] |
| CV | 21,39 | 23,90 |

*Means followed by the same letters within the same row are not significantly different at 5% level of LSD.*

However, if *Foc* isolates VCG 01213/16 co-infected with VCG 0120, percentage of wilt decrease until 26,6% and significant different on single inoculation of *Foc* isolate VCG 01213/16 to root tissue. The decline of wilted plant may be caused by the competition mechanism that occured between *Foc* isolate at initial infection tissue. The mechanism is for obtaining initial infection and for obtaining such nutrient [28].[29] stated that the fungus compete in the soil for oxygen. Some studies mentioned that mechanism of this competition occurs between strains of pathogenic and non-pathogenic Fusarium [3].

Refer to the progresion of wilted plant after four weeks, the percentage of wilted reach up to 20% on single or co-inoculation of *Foc* isolate VCG 01213/16 and VCG 0121 while others is lower than 20% (Figure 1). After four weeks the percentage of wilted plant from single inoculation and co-inoculation of *Foc* isolate VCG 01213/16 increase significantly and constantly since 5 weeks to 8 weeks. Toxin metabolites were produced by the fungus contributed to pathogenesis and plant invasion by *F.oxysporum* [27.30]. Furthermore, fusaric acid was produced by Fusarium hampered catalyzed enzyme, thus disturbing cell

membrane and causing ion leakage [31]. These pathogen has produced metylesterase and polygalactorase and others enzyme for ruining cell wall. Production of toxin caused water transportation obstructed, thereby instigating wilt and plant death [27. 32].

Analysis of the VCG appearance mentioned in the table 2 indicated that when co-inoculation *Foc* isolate VCG 01213/16 with the VCG 0120, 0121 and 01219 to the plant, *Foc* isolate VCG 01213/16 can compete absolutely against other VCG with percentage of VCG appearance up to 100%. These results strengthen the evidence that *Foc* TR4 isolate VCG 01213/16 is the most ferocious tropical pathogen, as well found by [26]. Race 4 of *Foc* is a strain attacking all banana varieties [14.21.33], as matter of fact that this race as hazardous *Foc* strain attacking banana in tropical area [34]. Furthermore, [35] mentioned that only the most competitive pathogens is able to colonize the wheat spike and cause Fusarium Head Blight.

Variability of virulence of several VCGs *Foc* on Ambon hijau cultivar can be attributed with variable of incubation period. Incubation period of each VCGs on Ambon hijau cultivar is varied significantly. Range of incubation period of single and co-inoculated *Foc* VCGs are 23,44 to 45,61 days. The the fastest incubation period of disease is on which inoculated with VCG 01213/16, while the longest is on plant was inoculated with isolate of *Foc* VCG 01219 (Table 2). The difference in incubation period of VCG 01213/16, 0121, 0120 and 01219 correlate to mechanism of plant defense. This condition shows that each VCG and host interact particularly, such [19.21] indicated that the relationship between virulence of a strain of *Foc* and plant host is very specific. It can be seen from table 2 that co-inoculation of *Foc* isolates VCG 01213/16 with VCG 0121 or with VCG 01219 results incubation period of disease as well on single inoculation of isolates *Foc* VCG 012131/6 with the incubation period ranges between 24.67 to 29 days after inoculation. However co-inoculation *Foc* isolates VCG 01213/16 with VCG 0120 delayed the incubation period until 10 days. It was assumed that the length of the incubation period on co-inoculation of *Foc* VCG 01213/16 isolates with VCG 0120 was caused by the *Foc* isolates VCG 0120 is able to be recognized by the plant earlier than *Foc* isolates VCG 01213/16 and direct reaction of plant defense system block the invasion of the pathogens, so that eventually *Foc* isolates VCG 01213/16 takes time to invade the plant.

In addition to incubation period or disease incidence, virulence of a pathogen was determined by the disease severity. Severity of emerging infectious disease was determined by a proportion of infected plant [25.26].

The highest disease severity index on leaves was on plant infested by *Foc* isolates VCG 01213/16 and *Foc* isolates VCG 0121, as well on co-inoculation *Foc* isolates VCG 01213/16 with VCG 0120 and VCG 0121, while the lowest was on single inoculation *Foc* VCG 01219 to plant. Correspondingly with the disease severity on corm, the highest disease severity index was found on the leaves which is inoculated with *Foc* isolates VCG 01213/16 and VCG 0121, and it was not significantly different with co-inoculation *Foc* isolates VCG 01213/16 with VCG 0120 and VCG 0121 (Table 3). The absence of differences in disease severity of leaves and corms of the plants which is inoculate single *Foc* isolates VCG 01213/16 or in coinoculated with other VCG proven malignancy of *Foc* isolates VCG 01213/16. According to [36]that the mycotoxin produced by each fungus hampers pathogen to colonize plant tissue.

## Conclusion

1. *Foc* isolates VCG 01213/16 is the most competitive strains among other VCG in race 4.

2. Result of VCGs analysis revealed that a VCG isolates were detected 100% from co-inoculation with other VCG.
3. Single or co-inoculation of *Foc* isolates VCG 01213/16 caused 69,6 to 94,8% plant wilted.
4. Co-inoculation of *Foc* isolates VCG 01213/16 with VCG 0120 delayed the incubation period of diseases until 10 days and decreased the percentage of wilted plant until 26.6%.
5. Co-inoculation of*Foc* isolates VCG 01213/16 with VCG 0120 reduced the disease severity on leaves and corm and significant different with at single inoculation of *Foc* VCG 01213/16.

## References


1. D. Fravel, C. Olivain, and C. Alabouvette. *Fusarium oxysporum* and its biocontrol. *New Phytologist* 157:493-502.2003.
2. C. Olivain, C. Steinberg and C. Alabouvette. Evidence of induced resistance in tomato inoculated by nonpathogenic strains of *Fusarium oxysporum*. In: Manka, M, ed. Environmental biotic factors in integrated plant disease control. Poznan, Poland: T*he Polish Phytopathological Society*, 427 – 430. 1995
3. A. Eparvier, C. Alabouvette. Use of ELISA and GUS-transformed strains to study competition between pathogenic and non-pathogenic *Fusarium oxysporum* for root colonization. *Biocontrological Science and Technology* 4: 35 – 47. 1994.
4. J. Postma, and A.J.G. Luttikholt. Colonization of carnation stems by a nonpathogenic isolate of *Fusarium oxysporum* and its effect on *Fusarium oxysporum* f. sp. *dianthi*. *Canadian Journal of Botany* 74: 1841 – 1851. 1996.
5. R.H. Stover. Studies on Fusarium wilt of banana. VIII. Differentiation of clones by cultural interactions and volatile substances. *Can. J. Bot.*,vol. 40, pp. 1467-1471.1957.
6. N.Y. Moore, K.G.Pegg, R. N. Allen and J. A. G. Irwin. Vegetative compatibility and distribution of *Fusarium oxysporum* f. sp. *cubense* in Australia. *Austr. J. Expt. Agric.* 33: 792-802.1993.
7. K.G. Pegg, N.Y. Moore &S. Bentley, 'Fusarium wilt of banana in Australia; a review'. *Austr. J. Agric. Res*., vol. 47, pp. 637-650. 1996
8. C. Hermanto, A. Sutanto, Jumjunidang, HS. Edison, J.W. Danniels, W. O'Neil, V.G. Sinohin, A.B. Molina &P. Taylor. Incidence and Distribution of Fusarium Wilt Disease in Indonesia. *In* Van den Bergh, I, Smith, M, Swennen, R, Hermanto, C. (*Eds*),*Proc*. *International ISHS-Promusa Symposium on Global Perspective on Asian Challenges. ISHS Acta Horticulture*, 828 p. 2011.
9. Jumjunidang, Riska&A. Soemargono.Identification and distribution of *Fusarium oxysporum*f. sp. *cubense*isolates through analysis of vegetative compatibility group in Lampung Province, Indonesia..*ARPN Journal of Agricultural and Biological Science*, vol. 7, no. 4, pp. 279-284. 2012.
10. M. Visser, T. Gordon, G. Fourie and A. Viljoen. Characterisation of South African isolates of *Fusarium oxysporum*f.sp. *cubense* from Cavendish bananas. *S.Afr. J. Sci.* 106(3&4): 1-6.2010
11. R.H. Stover. Studies on Fusarium wilt of banana. VIII. Differentiation of clones by cultural interactions and volatile substances',*Can. J. Bot.*,vol. 40, pp. 1467-1471.1962
12. A. Viljoen. 'The status of Fusarium wilt (Panama disease) of banana in South Africa', *South Africa J. Sci*., vol. 98. pp. 341-344. 2002.
13. S.C. Hwang, and W.H. Ko. Cavendish banana cultivars resistant to Fusarium wilt acquired through somaclonal variation in Taiwan.*Plant Disease*, vol. 88, pp. 580-588.2004.
14. R.C. Ploetz, and K.G. Pegg. Fusarium wilt of Banana a Wallace's Line: was the diseases originally restricted to his Indo-Malayan region?'*Australas. Plant Path*, vol. 26, pp. 239-249. 1997.
15. R.K.M. Thangavelu,Kumar,P.G. Devi &M.M Mustaffa. Genetic diversity of *Fusarium oxysporum* f. sp. *cubense* isolates of India by Inter Simple Sequence Repeats (ISSR) analysis. *Mol. Biotechnol*., vol. 51, pp. 203-211.2011.
16. Nurhadi, M. Rais, danHarlion. Seranganbakteridancendawanpadatanamanpisang di provinsiDati I Lampung. *Info Hort*. Vol. 2(1):37-40.1994.
17. N. Nasir, Jumjunidang dan Riska. Deteksi dan Pemetaan Distribusi*Fusarium oxysporum* f.sp.*cubense*pada Daerah Potensial Pengembangan Agribisnis Pisang di Indonesia. *J. Hort*. 5(1) : 50-57.2005.
18. J.F. Leslie. Genetic exchange within sexual and asexual populations of the genus Fusarium. *Dalam Fusarium* wilt of banana. APS Press. St. Paul, MN. 55-62.1990.
19. R.C. Ploetz. Variability in *Fusarium oxysporum*f.sp. *cubense*. *Can. J. Bot*. 68:1357-1363.1990.
20. .K.G. Pegg, R.G. Shivas, N.Y. Moore, and S. Bentley. Characterization of a unique population of *Fusarium oxysporum* f. sp. *cubense* causing

Fusarium wilt in Cavendish bananas at Carnavorn, Western Australia',*Austr. J. Agric. Res*.,vol. 46, pp. 167-178. 1995

21. H.J.Su, S.C. Hwang, and W.H Ko. Fusarial wilt of Cavendish bananas in Taiwan',*Plant Dis*., vol. 70, pp. 814-818.1986.
22. A.A. Mohamed, C. Mak, K.W. Liew, & Y.W. Ho. Early Evaluation of Banana Plants at Nursery Stage of Fusarium Wilt Tolerance. *In*: Molina, AB, Nik Masdek, NH & LiewKW (*Eds*.) Banana Fusarium Wilt Management: Towards Sustainable Cultivation,*Proceedings of The International Workshop on Banana Fusarium Wilt Diseases*. Malaysia. INIBAP,pp. 174-185. 1999
23. P.A. Pittaway, N. Nasir and K.G. Pegg. Soil receptivity and host pathogen dynamic in soils naturally infested with *Fusarium oxysporum*f. sp.*cubense*, the cause of Panama disease in bananas. *Austr. J. Agric. Res*. 50:623-628.1999.
24. R.H. Stover. Plantain and Abaca Diseases. Commonw. Mycol. Inst. Kew, Surrey. England. 319 pp.1972.
25. D.R. Jones. Technical Guidelines for IMTP Phase II: Fusarium Wilt Sites',*In*: The Improvement and Testing of Musa: a Global Partnership, *Proceedings of the first conference of the international Musa Testing Program*, helt at FHIA, Honduras. INIBAP,pp. 279-286. 1994
26. Riska, Jumjunidangdan C. Hermanto. Hubungantingkatkonsentrasiinokulum*Fusarium oxysporum* f. sp. *cubense* VCG 01213/16 denganperkembanganpenyakitlayupadakultivarpisa ngrentan. *JurnalHortikultura* Vol 22 (2) ; 156-164 tahun 2012
27. S. Groenewald. Biology, pathogenicity and diversity of *Fusarium oxysporum f. sp. cubense*', *Requirement for the Degree of Magister Scientiae In the Faculty of Natural and agricultural Science, Universiyt of Pretoria*, Pretoria, 176 p.2005.
28. C. Alabouvette, and Y. Couteaudier. Biological control of fusarium wilts with nonpathogenic Fusaria. In: TjamosEC, CookRJ, PapavizasGC, eds. Biological control of plant diseases. New York, USA: Plenum Press, 415–426.1992.
29. Z. L1, N. Takaya N, M.A. Sakairi, H. Shoun.Oxygen requirement for denitrification by the fungus *Fusarium oxysporum.*Arch Microbiol 175(1):19-25. 2001
30. X LI, T. Bai Y. Li, X. Ruanand H. Li. Proteomic analysis of *Fusarium oxysporum* f. sp. *cubense* tropical race 4-inoculated response to Fusarium wilts in the banana root cells. Proteome sci. 11;41. 2013
31. A. Balio. Structure activity relationship. New York Academic Press.1981.
32. L. SamadiandB.S. Behboodi .Fusaric acid induces apoptosis in saffron root-tip cells: roles of caspase-like activity, cytochrome c, and H2O2. *Planta*, *255*(1), 223–234.2006.
33. R.H. Stover and I.W. Buddenhagen., 'Banana Breeding: polyploidy, disease resistence and productivity', *Fruits*, vol. *41*, pp. 175-191.1986
34. A. Daly &G. Walduck. Fusarium Wilt of Bananas (Panama disease) (*Fusarium oxysporum*f. sp. *cubense*)'. *Agnote*. vol. I51, 5 p.2006
35. D. Siou, S. Gelisse, V. Laval, S. Elbelt, C. Repincay, M. Bourdat-Deschamps, F.Suffert, and C. Lannou. Interactions between head blight pathogens: consequences on disease development and toxins production in wheat spikes. Appl.EnvironMicrobiol 81:957-965. 2015.
36. X.M. Xu, P. Nicholson, M.A. Thomseti, D. Simpson, B.M. Cooke, F.M. Doohan,J. Brennan, S. Monaghan, A. Moretti, G. Mule, I. Hornok , E. Beki, J. Tatnell, A. Ritieni, S.G. Edwards.. Relationship between the fungal complex causing Fusarium head blight of wheat and envinromental consitions93:878. 2009

# Changes nutrients by microbial fermentation chocolate waste indigenous result of the additional mineral phosphor and sulphur in-vitro

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## Abstract

Waste chocolate consists of leaves and rind that can pollute the environment therefore be used as an alternative feed cattle the way fermentation using microbes indigenus sellulotik isolated beforehand, aims to see the extent of mineral Sulpur and phosphorus can increase the nutrients from the leaves brown fermented with microbes indigenus cellulolitic. Fermentation is a process that is suitable for processing on high-fiber feed ingredients. This study uses a completely randomized design with three replications faktorialdengan patterns. Treatment of this study consists of factor A is the waste brown, A1 = rind brown A2 = leaves are brown and factor B is microbial indigenus, B1 = MI rind brown, B2 = MI brown leaf, B3 = mixture of MI rind (50% ) and brown leaf MI (50%). In this study Sulpur added mineral and mineral phosphorus as much as 0.01%. The research variables are food substances in the form of dry matter, organic matter, crude protein, crude fiber, NDF, ADF, hemicellulose and cellulose. The results obtained from the addition of mineral sulpur and phosphorus into the waste fermentation using microbes indigenus chocolate can improve the nutritional content of chocolate waste Increased dry matter content of about 44.89%, 47.08% crude protein and crude fiber 38.78%.

Keywords: waste, brown, mineral, fermentation

## 1. Introduction

Livestock farms ruminants is a commodity the potential to be developed to meet the needs of the meat to the public. The productivity of ruminants in general remains low, this is due to the fluctuations in the availability of feed quality is poor and grass land conversion into agricultural land and buildings. To overcome the problem of the utilization of waste need plantation chocolate brown as the leaves are trimmed regularly so if not in use will environment pollution. Brown leaves are fermented by microorganisms indigenus has a dry matter content of 15-17%, 5-6% crude protein, crude fiber 20-23% and ADF content of 49.79%, 24.33% lignin (Fridarti, 2013).

Supplementation can be classified into two parts, namely a complete nutrient supplement containing energy, protein, and minerals as well as functional feed supplement that works to improve feed efficiency and as a growth promoter for the rumen microbes. Both types of these supplements will be to increase the capacity of livestock digest for their metabolism and repair capabilities rumen microbes and ultimately improve livestock productivity.

Nutrient supplements derived from local materials which are abundantly available like coconut cake, bran, cassava leaves, calliandra, cassava, essential minerals such as Ca, P and S. nutrient supplements required for the rumen microbes need nutrients that are easily available is used for growth such as energy, protein, and minerals. In accordance with the opinion of Leng (1991) in which the increase in the digestibility of feed fiber must also be

approached in terms of the adequacy of nutrients for rumen microbial growth. Optimal microbial growth requires sufficient nutrients in the rumen such as energy, protein, amino acids, and minerals. Minerals also added importance due to the feed in the tropics and feed derived from agricultural waste or plantations are often deficient in minerals essential to the growth of microbes such as P and S, (Preston and Leng, 1987; Komisarczuk and Durand, 1991), and added again that bioavailability of minerals in feed is also low in fiber and supplementation is able to improve feed digestibility fiber (Little, 1986; Mardiati Zain et al, 2010a, 2010b).

Functional feed supplement is also needed in order to improve the efficiency of feed utilization. Functional feeds that will be tested, namely the production of methane gas are lower, defaunasi agents to control the population of rumen protozoa and the addition of probiotics aimed at improving the working population and rumen microbial enzymes. The second type of supplementation will be tested right formulation to improve the digestibility and rumen microbial population that will be the impact on increasing the productivity of livestock.

Based on the description above to spur high livestock produksitivitas overall approach is necessary. Bioprocess rumen strongly influenced by Fermentability feed and microbial activity. Thus a blend of processing technology with the addition of supplements, especially on the use of feed fiber waste junky like brown rind will give great hope to increase the productivity of cattle. Objective to see how far their waste makanandari-enhancing substance brown (brown rind, brown leaf)

## 2.Material and Methods

This study uses a plantation in the form of fruit skin brown, brown leaf with the method of fermentation using microorganisms isolates, which kosentrasinya is 15% of the substrate, and then added with a solution of sugar 10%, and the feces of chicken as much as 2% of dry matter, mineral sulfur (S) and phosphorus (P) as much as 0.1% and then stored for 6 days later the results obtained will be finely ground to see the food substance content of each ingredient with methods of proximate (Tilley and Terry, 1963).

This study uses a completely randomized design pattern factorial with three replications which factor A is a type of agricultural waste (A1 = rind brown, A2 = brown leaf.) Factor B is a type of microorganism (B1 = Microorganisms origin of the fruit skin brown (KBC), B2 = MO brown leaf origin (DC), B3 = mixture MO (50% Mo + 50% KBC origin origin DC) Parameters measured in this study are: 1.Kandungan dry matter, organic matter, Crude Protein and Crude Fiber 2.Kandungan NDF, ADF, hemicellulose, cellulose

Measurement data variables in the analysis: analysis of variance, and when there is a very real influence then did a further test with LSD

## 3.Result and Discussion

Results addition of mineral Sulpur and phosphorus in the waste cocoa fermented with microorganisms indigenus can be seen in Table 1. The results of the study of waste cocoa fermentation with microbes indigenus yag coupled with mineral sulpur and phosphorus has a content of dry matter ranged from 22.228 to 30.849%, organic matter content from 69.021 to 78.050%, crude protein from 7.628 to 8.825% and crude fiber from 24.588 to 31.881%., While NDF content of 80.866 to 87.646%, ADF 60.847 to 68.231%, hemicellulose 10.509 to 22.277%, cellulose 37.315 to 44.558%.

These results are higher than research Fridarti (2013) in which the dry matter content of brown leaf fermentation with micro-organisms indigenus is 15-17%, crude protein 5-6%, crude fiber 20-23%, 49.79% ADF.

Table 1. The contentbrown waste food substances fermented by microorganisms in the added indigenus the Mineral S and P

| content | Microbial indigenous | | | Mean |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| | | Dry matter | | |
| Fruit Leather | 24.117[a] | 22.228[a] | 22.941[a] | 22.877[A] |
| Brown leaves | 29.978[c] | 30.849[d] | 27.496[b] | 29.830[B] |
| Mean | 26.973[A] | 26.507[A] | 25.250[C] | |
| | | Organic materials | | |
| Fruit Leather | 75.883[c] | 77.772[c] | 78.059[c] | 77.123[A] |
| Brown leaves | 70.473[ab] | 69.021[a] | 72.759[b] | 70.339[B] |
| Mean | 73.177 | 73.449 | 75.454 | |
| | | Crude protein | | |
| Fruit Leather | 7.628[a] | 8.557[b] | 8.615[b] | 8.736 |
| Brown leaves | 8.215[b] | 8.766[b] | 8.825[b] | 8.911 |
| Mean | 8.438 | 8.632 | 8.799 | |
| | | Crude fiber | | |
| Fruit Leather | 24.588[a] | 31.881[b] | 31.116[b] | 30.200 |
| Brown leaves | 35.802[c] | 31.733[b] | 31.135[b] | 32.179 |
| Mean | 28.745[A] | 31.762[B] | 31.445[B] | |
| | | The content of NDF | | |
| Fruit Leather | 80.866[a] | 84.159[b] | 87.646[c] | 84.561 |
| Brown leaves | 85.279[b] | 87.292[c] | 80.948[a] | 82.401 |
| Mean | 82.741 | 85.763 | 83.007 | |
| | | The content of ADF | | |
| Fruit Leather | 60.847[a] | 61.881[a] | 68.231[c] | 62.611 |
| Brown leaves | 65.298[b] | 60.237[a] | 66.596[b] | 62.339 |
| Mean | 63.389[B] | 60.977[A] | 67.366[C] | |
| | | The content of Hemicellulose | | |
| Fruit Leather | 20.019[b] | 22.277[b] | 19.415[b] | 20.950[B] |
| Brown leaves | 10.509[a] | 12.138 | 11.207[a] | 12.225[A] |
| Mean | 15.214 | 17.217 | 15.311 | |
| | | The content of Cellulose | | |
| Fruit Leather | 37.315[a] | 40.399[ab] | 44.558[c] | 40.508 |
| Brown leaves | 38.579[a] | 39.853[ab] | 39.802[ab] | 40.508 |
| Mean | 39.244 | 38.677 | 41.951 | |

Description: a, b, c, .. superscript in columns and rows of different shows highly significant effect ($P < 0.01$); A, B, C superscript in different columns or rows that show a significantly different effect ($P < 0.05$)

The content dry material brown rind results of this study are higher than the results BPTP (2007) that fermented brown rind that use of probiotics containing approximately 18.4% dry matter. As well as results of research Erna (2004) which uses Bacillus sp in the fermentation of fruit skin brown is 15.28%.

Based on the statistical test results of analysis of variance showed that the interaction between microbes indigenus with the addition of mineral S and P showed highly significant effect

(P <0.001) to reduce the content of dry matter, crude fiber and increase organic matter, crude protein waste chocolate. This is presumably the effect of adding mineral Sulpur and Phosphorous against indigenus microbial activity in the fermentation process, it can cause an increase in the nutrient content of the leaves and bark brown fermented fruit. These results are in accordance with the opinion of Preston and Leng (1987); Komisarczuk and Durand (1991) that the added minerals are also important due to the feed in the tropics and feed derived from agricultural waste or plantation, feed derived from sewage is often deficient in minerals essential to the growth of microbes such as P and S. Added by Little, ( 1986); Mardiati Zain et al, (2010a, 2010b) that the bioavailability of minerals in feed is also low in fiber and supplementation is able to improve feed digestibility of fiber.

## Conclusion

The addition of mineral Sulpur and phosphorus can increase the content of nutrients from the leaves and bark brown fruit, an increase of about 44.89% dry matter, crude protein 47.08%, 38.78% crude fiber.

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## References

Hall Pengakajian Agricultural Technology (BPTP) Lampung. 2007. Fruit Leather Cocoa as Feed. Bandar Lampung.

Fridarti, 2011. Improved Nutritional content of fruit skin brown fermented with Local Microorganisms (MOL) brown rind. Jur, Vol.11 # 2 terms: 61-66

Fridarti, 2012. Applications Fermented Fruit Leather Brown to improve the quality of animal feed in Nagari Lurah Ampalu. Jur. Ektronas, vol 12 2 terms: 57-62

Fridarti, 2013. The level of digestibility of nutrients from fruit Kulut Brown fermented by microorganisms indigenus. Jur. Science towers vol.X No. Case 43: 36-41.

Fridarti, 2014. Effects of Fruit Leather Brown fermentation with micro- organisms indigenus against digestibility BK, BO, PK sheep livestock rations. Higher Education Competitive Grant Research Report, 2014.

Komizarczuk, S., Durand M. 1991. Effect of minerals on microbial metabolism. In. Rumen Microbial Metabolism and Digestion ruminants. J.P. Jouany (Ed) INRA Publ. Versailles, France.

Leng, R.A. Application of Biotechnology to 1991 of Animal Nutrition in Developing countries. FAO Animal Production and Health Paper.

Little, D.A. 1986. The mineral content of ruminants feed and the potential for mineral supplementation in South - East Asia with particular reference to Indonesia. In. R.M. Ed Dixon. IDP. Camberra.

Mardiati Zain, N Jamarun and Nurhaita, 2010a. Effect of Sulfur Supplementation on in vitro Fermentability and degradability of ammoniated Rice Straw. Pakistan Journal of Nutrition 9 (5): 413-415, 2010.

Mardiati Zain, N. Jamarun and Zulkarnaini. 2010b. Effect of phosphorus and sulfur supplementation in growing beef cattle diets based on rice straw ammoniated. Asian Journal of Scientific Research 3 (3): 184-188. 2010.

Mardiati Zain, N. Jamarun and Elihasridas. 2002. Supplementation grass with straw processed into cattle feed. J.Andalas. 31 / May / Year XIV / 2002.

Preston, T.R. and R.A. Leng. Matching ruminants 1987. Production Systtem with Available Resources in the Tropics. Conjurer Books. Armidale.

Tilley, J. M. A. and R. A. Terry. 1963. A two-stage technique for the in vitro digestion of forages crop. J. Br. Grass. Soc.

# Clustering and genetic distance some salak species (*Salacca* spp) based on morphological characters

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## Abstract

The objectives were to determine clustering and genetic distance some salak speciesand their crossing based on morphological characters. The research was conducted at Aripan Experimental station of Indonesian Tropical Fruit Research Institute, Solok from June 2012 to May 2013. Materials genetic were 30 accessions which be composed of 8 years old salak species and their crossing. Each accession consisted of one plant which be derived from generative propagation. The data obtained from characterization were analysed with NTSYS program version 2.02. The result showed that based on morphological characters, 30 salak accessions can be clustered into 8 groups at 52% genetic similarity. At this similarity, Gading Bali*(Salacca zalacca* var Amboniensis), Sidempuan (*S. sumatrana*), *S. Affinis, S. walliciana, and S. glabrescens* were in separate groups. Species which had the smallest genetic similarity (21.74%) were between Gula Pasir ( *S. zalacca* var *amboinensis*) or Pondoh *(S. zalacca* var. *zalacca)* and *S. glabrescens* or *S. Affinis;* likewise between *S. sumatrana* and *S. affinis*. Thisinformationcan be used for consideration in the selection of parents to produce superior variety.

Keywords: Clustering; genetic distance; morphological character; *Salacca* spp.

## 1. Introduction

In Indonesia, salak (*Salacca* spp) has a competitive advantage is compared to other countries. It has high diversity in genetic resource spreading in most provinces of Indonesia. There are 20 species of salak in the world which are distributed in Indo Malaysia region namely Assam, Burma, Siam, Indo-China, Malaysia, Sumatera, Borneo, Java, Bali, and the Philippines. Salak has been planted in Queensland (Australia), New Guinea, Ponape island and Fiji island (e.g. [1], [2]).

The fruit contains vitamins and minerals which is useful for healthy. Each 100 grams of fruit contains 77 calories, 0.5 grams proteins, 20.9 grams carbohydrates, 28 miligrams calciums, 18 miligrams phosphorus, 4.2 miligrams irons, 0.04 miligrams vitamin B, and 2 miligrams vitamin C (e.g. [3]). This fruit also has been an income source of farmers in the production centers in Indonesia. It can be eaten in the process form such as candies, chips, pickles, wine and others, so it can be stored in the long time. By extending the shelf life, it can be expanded marketing and its product can be guaranteed throughout the year.

Indonesia is rich in genetic diversity. From 20 species of *Salacca* spp that exist in the world, 13 species are found in Indonesia which spread in Sumatera, Java, Bali, and Kalimantan. Three species of them are tasty to be eaten. They are *Salacca zalacca, S. Sumatrana* and *S. Affinis*(e.g. [4]). Diversity species with different characters are genetic source for germplasm collection and to producesuperior variety.

Genetic markers which can be used to distinguish in intra and inter species are morphological marker, agronomic, and molecular. Morphological marker is easier, fast, simple, and cheap. It can be used to analysis of phylogenetic relationship, and also knowing genetic distance among accessions; however, the weakness of morphological marker is some characters are influenced by environment. It is necessary to choose the characters that have high heritability and stable (e.g. [5], [6]). The differences and similarities morphological of a plant can be used to find phylogenetic relationship(e.g. [7], [8]).

To determine the parental crossing, It needs wide phenotypic and genotypic variabilities, descriptions, genetic distance, and phylogenetic relationship informationsof the parent which will be crossed. The farther of genetic distance betweenparents will obtain a hybrid with high heterosis (exceeding both parents) (e.g. [9], [10]).

Table 1. The accessions list which be used in the research

| No | Accession | Explanation |
|---|---|---|
| 1 | 50 - 12 PH-K | Hybrid |
| 2 | 48 - 21 PH-K | Hybrid |
| 3 | 50 - 7 PH-K | Hybrid |
| 4 | 49 - 19 PH-M | Hybrid |
| 5 | 17 - 6 PH-MJ | Hybrid |
| 6 | 1 - 27 PH-MJ | Hybrid |
| 7 | 41 - 7 PH-SJ | Hybrid |
| 8 | 44 - 3 SDM | *Sidempuan (S. Sumatrana* |
| 9 | 45 - 28PMGJC | Hybrid |
| 10 | 38 - 22 SS-SJ | Hybrid |
| 11 | 36 - 15 SS-SJ | Hybrid |
| 12 | 11 - 9 MW-SP | Hybrid |
| 13 | 11 - 18 MW-SP | Hybrid |
| 14 | 11 - 3 MW-SP | Hybrid |
| 15 | 23 - 22 MW-SP | Hybrid |
| 16 | 27 - 18 MWR | Mawar |
| 17 | 26 - 14 MWR | Hybrid |
| 18 | 12 - 14 MW-AFN | Hybrid |
| 19 | 12 - 4 MW-AFN | Hybrid |
| 20 | 23 - 3 SBGP | Gula Pasir *(S. zalacca* var *amboinensis)* |
| 21 | 24 - 18 SBGP | Gula Pasir *(S. zalacca* var *amboinensis)* |
| 22 | 18 - 11 SJG | Sanjung |
| 23 | 19 - 27 SL-SJ | Hybrid |
| 24 | 20 - 5 JW-PH | Hybrid |
| 25 | Glabrescens | *S. glabrescens* |
| 26 | Affinis | *S. affinis* |
| 27 | Gading Bali | *S. zalacca* var *amboinensis* |
| 28 | Wallichiana | *S. wallichiana* |
| 29 | 7 - 6 PH-JY | Pondoh *(S.zalacca* var. *zalacca)* |
| 30 | 7 - 8 PH-JY | Pondoh*(S.zalacca* var. *zalacca)* |

The studies of genetic diversity of salak have been carried out by [11], who worked on genetic diversity, genetic distance and identification of *Salacca zalacca* in Java based on RAPD; and [7] who worked on morphological study and phylogenetic relationship salak Pondoh cv. based on morphological character. The genetic distance information and phylogenetic relationship among salak species have not been reported widely. This study purpose was to determine the clustering and genetic distance some salak species and its crossing based on morphological characters.

## 2. Material and Methods

The research was conducted from June 2012 to May 2013 at Aripan Experimental station of Indonesian Tropical Fruit Research Institute, Solok.Its altitude is 413 meters above sea level.

The characterization was done on 30 accessions from some salak species and their crossingswhich be composed of 8 years old (Table 1). All these accessions were grown from seeds with planting distance of 3 m x 3 m. Every accession consisted of one plant.

The characterization was done by individual plant. Every plant was given a number and observed twice in the harvest season. The characters which be used to determine the cluster and genetic distance were morphological characters, namely qualitative and quantitative. The characterization was done by guidelines testing individual of salak [12]. The qualitative characters were immature young leafcolor, leaf upper surface color, leaf lower surface color, thickness of wax on leaf lower surface, petiole color,revolute, leaf margin spine color, spine shape, presence of spine on fruit, flesh color, astringent taste on flesh. Quantitative characters were petiole length (cm), distribution of spines at leaflet margin ,leaflet length (cm), leaflet width (cm), top part of leaf length (cm), top part of leaf width (cm), spine length (cm ), fruit diameter (cm), fruit weight (g), flesh thickness (cm), Total Soluble Solid (TSS) of flesh (˚ Brix). Data were analyzed with NTSYS program version 2.02 with the final result was dendogram.

## 3. Result and Discussion

Based on dendogram, 30 accessions can be grouped into 8 groups at 52% genetic similarity (Figure 1). The greater values of genetic similarity coefficient indicated that genetic distance values was smaller (genetic distance was close).

Group I consisted of 7 accessions. All these accessions were hybrids which female parent was Pondoh and male parent was *Salacca zalacca* var. *zalacca* species. The characteristics of this group were dark green color at leaf upper surface, leaflet margin was not revolute, brown spine color, yellow white 158A flesh color, TSS>19˚brix, and astringent taste. Between accession41-7-Ph-Sjg and 48-21-Ph-K had the smallest genetic similarity, it was 43.48% orgenetic distance was 0.57. In contrast, accession 49-19-Ph-Mj and41-7-Ph-Sjg and between accession 49-19-Ph-Mj and 50-12-Ph-K had the greatest genetic similarity, it was 73.91% orgenetic distance was 0.26.

Among 8 groups were formed, group II had the most many members, namely 14 accessions. The characteristics of this group were thick wax on leaves lower surface, spiny on an half of leaflet margin, 4.1 – 6 cm on leaflet width, 10.1 – 15 cm on top part of leaf width, 4.1 – 5 cm on fruits diameter, 19.1 – 21°brix on TSS, and astringent taste. This group was divided by two groups at 55% similarity genetic distance, i.e. group IIa and IIb. Group IIa consisted of 11 accessions, while group IIb consisted of 3 accessions. Differences these groups were group IIa had brown immature young leaf , black spine color, and 0.51 – 1 cm on flesh thickness, while group IIb had yellow green color on immature young leaf , brown spine color, 0.51 – 0.75 cm on flesh thickness. At group II, the

accession which had the smallest similarity level (35.56%) or genetic distance 0.64 was between 45-28-PMG-Jc and 18-11-Sjg, while the biggest similarity genetic (82.61%) or genetic distance 0.17 was between 20-5-Jw-Ph and 7-6-Ph-Jy.

Salak Mawar (Mwr) and Gula Pasir (SBGP) were in the same group, namely group III. Salak Mawar (Mwr) was hybrid, , one of the parent was Gula Pasir which belongs to species (*Salacca zalacca* var. *Amboinensis*). The special characteristics of this group were revolute on leaves margin, black spines color, 19 – 21°brix on TSS, not astringent taste and white color (white NN 155A) on flesh.

Group IV consisted of one accession, namely Gading Bali. It was included in Bali species (*Salacca zalacca* var. *amboinensis*). Morphological characters of Gading Bali were almost similar to Gula Pasir, except for brown yellowish on skin, Yellow white 158 A on flesh, thick flesh (1.13 cm), and it felt slightly astringent. Gading Bali had the greatest genetic similarity (60.87%) or genetic distance was 0.39 with accession 12-14-Mw-AFN and the smallest genetic similarity (26.09%) or genetic distance was 0.74 with accession 17-6-Ph-Mj and 23-22-Mw-Sp.

Accession 44-3 Sidempuan was in group V. This accession was included in *Salacca sumatrana* Becc. It was found in North Sumatera region [2]. Specific characteristics of this species were it has larger size plant than salak Jawa (*Salacca zalacca* var. *zalacca*.) and Bali (*Salacca zalacca* var. *amboinensis*). Reference [13] showed that Sidempuan has greater size on tree height, petiole length, andtop part of leaf width than Pondoh and Gula Pasir. According to [14] top part of leaf length and top part of leaf width can be used to differentiate among salak species. At this research, accession 44-3 Sidempuan were 87.67 cm on leaflet length, 8.10 cm on leaflet width, 56.67 cm on top part of leaf length, 19.77 cm on top part of leaf width and it relatively larger than the other accessions. Accession 44-3-Sidempuan had the smallest genetic similarity (21.74%) or genetic distance was 0.78 with accession *S.affinis* and the greatest genetic similarity with accession 38-22-SS-Sj; 36-15-SS-Sj; and 17-6 PH-MJ was 47.83% or genetic distance was 0.52.

*Salacca wallichiana* wasin group VI. This species had some advantages, i.e. spineless on petiole, the bunch had many fruits (> 300 fruits per bunch) and its taste was not astringent, so this species is potential as an parental plant in combination of new varieties. However, its weakness was very sour taste, soft texture of flesh, and thin flesh. This species had the smallest genetic similarity (17.78%) or genetic distance with 1-27-Ph-Mj was 0.82 and the largest genetic similarity with 44-3-Sidempuan was 44.44% or genetic distance was 0.56.

*Salacca glabrescens* was in group VII. This species had specific characteristics. There were no wax on leaf lower surface, yellow orange 20D on flesh color, and low TSS (13.53°brix). This species had the smallest genetic similarity (17.39%) or genetic distance was 0.83 with 17-6-Ph-Mj and the greatest genetic similarity with 11-9-Mw-Sp, it was 39.13% or genetic distance was 0.61.

Group VII consisted of a single species that was *Salaccaaffinis.* This species had special characters, i.e. spineless skin, but the weakness flesh were soft texture, juicy, and astringent. This species had the smallest genetic similarity with 11-18-Mw-Sp, it was 8.70% or genetic distance was 0.91, the greatest genetic similarity to species *S. glabrescens* by 52.17% orgenetic distance was 0.48.

Based on this dendogram, the accessions which had the greatest genetic similarity (82.61%) was between 20-5-Jw-Ph and 7-7-Ph-Jy. To improve the effeciency of using and maintenance of germplasm, so we

select one accession from representative group. Among the species which be observed, the species that had the smallest genetic similarity (21.74%) were between Gula Pasir (*S. Zalacca* var. *amboinensis*) or Pondoh (*S. Zalacca* var. *zalacca*) and *S. Glabrescens* or *S. Affinis* and between *S. Sumatrana* and *S. Affinis.* Species which have small genetic similarity or far genetic distance is better used as a parent for crossing to get high heterosis effect.

## Conclusion

Based on morphological characters, 30 accessions can be grouped into 8 groups at 52% genetic similarity. At this genetic similarity, Gading Bali (*S. Salacca* var *Amboniensis*), Sidempuan (S. *sumatrana*), S. *affinis*, S.*walliciana*, and S. *glabrescens* formed separate groups. Species that have the smallest genetic similarity (21.74) % were between Gula Pasir (*S. zalacca* var *amboinensis*) or Pondoh *(S. zalacca* var. *zalacca*) and *S. Glabrescens* or *S. affinis;* between *S. sumatrana* and *S. Affinis*.

## References


[1] Furtado C.X, "PalmaeMalesicae-x, TheMalayan SpeciesofSalacca.Gardens",Bulletin, SingaporeVol XII, part 2, pp. 378-403, December 1949.

[2] Verheij, E.W.M., and R.E. Coronel, *Edible fruit and nuts*, PROSEA, Bogor, Indonesia, 1992.

[3] Kusumo, S., F.A. Bahar., S. Sulihanti, Y. Krisnawati, Suhardjo, dan T. Sudaryono, *Teknologi Produksi Salak*, Pusat Penelitian dan Pengembangan Hortikultura, Jakarta, 1995.

[4] Mogea, J. P, "Beberapa aspek fenologi salak (*Salacca edulis* Reinw)", Thesis, Institut Teknologi Bandung, 1973

[5] Lamadji, S, "Pemberdayaan sifat morfologi untuk analisis kekerabatan plasma nutfah tebu", Bulletin P3GI. vol. 148, pp. 17-31, 1998.

[6] Hadiati, S., S. Yulianti, dan Sukartini, "Pengelompokan dan jarak genetik plasma nutfah nenas berdasarkan karakter morfologi", J. Hortikultura, vol. 19, no. 3, pp. 264-274, 2009.

[7] Suskendriyati,H., A. Wijayati, N. Hidayah, dan D. Cahyuningdari, "Studi Morfologi dan Hubungan Kekerabatan Varietas Salak Pondoh (*Salacca zalacca*(Gaert.) Voss.) di Dataran Tinggi Sleman", Biodiversitas, vol. 1, no. 2, pp. 59-64, 2000.

[8] Babic, M., V.Babic,S. Prodanovic,M. Filipovic, and V. Andjelkovic, "Comparison of morphological and molecular genetic distances of maize inbreds. Genetica", vol. 44, no. 1, pp. 119-128, 2012.

[9] Tatineni, V., G. Cantrell, and D.D. Davis, "Genetik diversity in elite cotton germplasm determined by morphological characteristics and RAPDs", Crop Sci, vol. 36, pp. 186-192, 1996.

[10] Bruel, D.C., V.C. Pipolo, A.C. Gerage, N.S.F. Junior, C.E.C. Prete, C.F. Ruas, P.M. Ruas, S.G.H. Seuza and D.D. Garbugilo, "Genetik distance estimated by RAPD markers and its relationship with hybrid performa Pesq.agropec.bras., Brasilia, vol. 41, no. 10, pp. 1491-1498, 2006.

[11] Nandariyah, Soemartono, W.T. Artama, DAN Taryono, "Keragaman kultivar salak (*Salacca zalacca*)", Agrosains, vol. 6, no. 2, PP. 75-79, 2004.

[12 Pusat Perlindungan Tanaman, "Panduan Pengujian Individual Tanaman Salak (*Salacca zallaca*). BUSS", Pusat Perlindungan Tanaman, Departemen Pertanian, 2006

[13] Hadiati, S., T. Budiyanti, dan A. Susiloadi, "Hasil persilangan dan pertumbuhan beberapa genotipa salak", Gading Baletin Plasma Nutfah 1, vol. 4, no. 1, pp. 26-32, 2008.

[14] Murti, R.H., D. Prajitno, A. Purwantoro, dan Tamrin, "Keragaman genotip salak lokal Sleman", J. Habitat, vol. 13, no. 1, pp. 57-65, 2002.

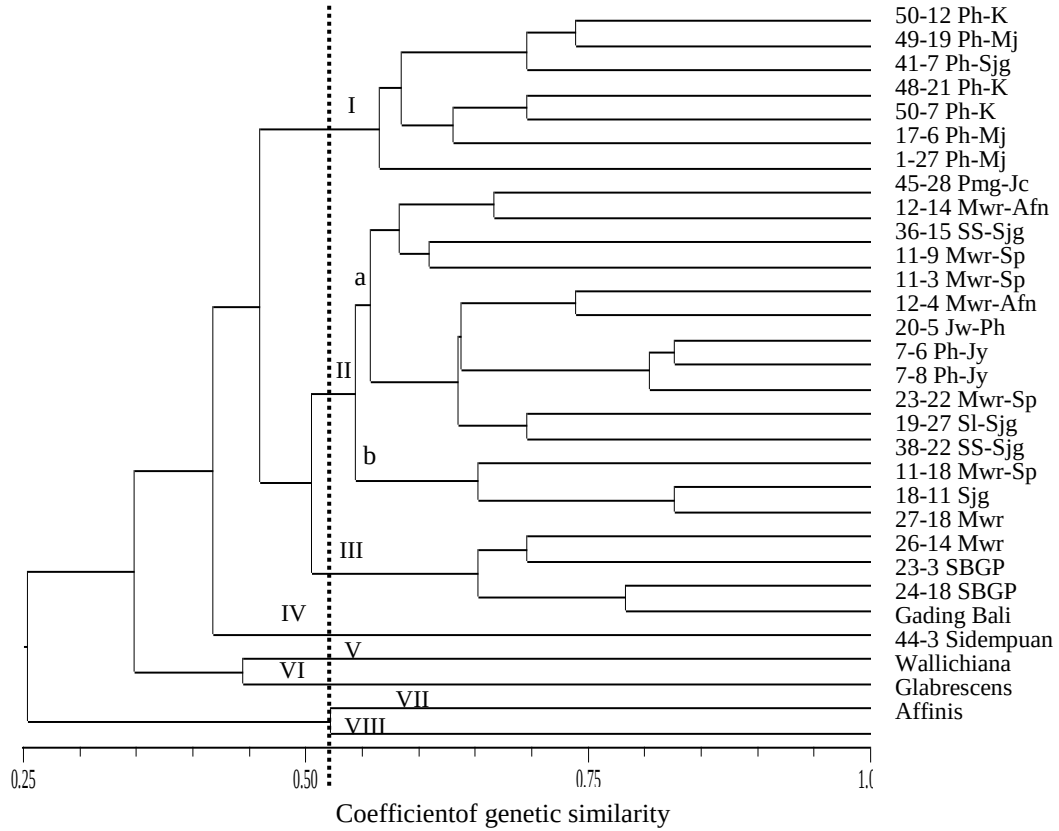


Figure 1. Dendogram of 30 accessions of salak based on morphological characters

Appendix 1.The similarity morphological matrix on 30 accessions of salak

| Aksesi | 50 - 12 PH-K | 48 - 21 PH-K | 50 - 7 PH-K | 49 - 19 PH-M | 17 - 6 Ph-Mj | 1 - 27 Ph-Mj | 41 - 7 Ph-Sj | 44 - 3 SDM | 45 - 28 PMGJC | 38 - 22 Ss-Sj | 36 - 15 Ss-Sj | 11 - 9 Mw-Sp | 11 - 18 Mw-Sp | 11 - 3 Mw-Sp | 23 - 22 Mw-Sp |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| 2 | 0.6522 | 1.0000 | | | | | | | | | | | | | |
| 3 | 0.6957 | 0.6957 | 1.0000 | | | | | | | | | | | | |
| 4 | 0.7391 | 0.6522 | 0.6087 | 1.0000 | | | | | | | | | | | |
| 5 | 0.4783 | 0.6087 | 0.6522 | 0.5217 | 1.0000 | | | | | | | | | | |
| 6 | 0.6522 | 0.5652 | 0.6087 | 0.5652 | 0.4783 | 1.0000 | | | | | | | | | |
| 7 | 0.6522 | 0.4348 | 0.5652 | 0.7391 | 0.6522 | 0.5217 | 1.0000 | | | | | | | | |
| 8 | 0.3043 | 0.3913 | 0.3913 | 0.4348 | 0.4783 | 0.3913 | 0.4348 | 1.0000 | | | | | | | |
| 9 | 0.4889 | 0.6667 | 0.4444 | 0.4444 | 0.4444 | 0.4444 | 0.3556 | 0.4444 | 1.0000 | | | | | | |
| 10 | 0.6522 | 0.4783 | 0.4783 | 0.4783 | 0.5217 | 0.4783 | 0.5217 | 0.4783 | 0.4444 | 1.0000 | | | | | |
| 11 | 0.6087 | 0.5217 | 0.4348 | 0.5652 | 0.4348 | 0.5217 | 0.5217 | 0.4783 | 0.6222 | 0.5652 | 1.0000 | | | | |
| 12 | 0.5652 | 0.4783 | 0.5652 | 0.3913 | 0.3478 | 0.4783 | 0.3478 | 0.4348 | 0.5333 | 0.5652 | 0.6087 | 1.0000 | | | |
| 13 | 0.5652 | 0.3043 | 0.3043 | 0.4783 | 0.4348 | 0.4348 | 0.5217 | 0.3913 | 0.4444 | 0.6957 | 0.5652 | 0.6087 | 1.0000 | | |
| 14 | 0.4348 | 0.4348 | 0.3913 | 0.3913 | 0.3478 | 0.4348 | 0.3043 | 0.3913 | 0.5778 | 0.5217 | 0.5652 | 0.6522 | 0.6957 | 1.0000 | |
| 15 | 0.6087 | 0.6087 | 0.5217 | 0.5652 | 0.5652 | 0.3913 | 0.4348 | 0.3043 | 0.6667 | 0.4348 | 0.6522 | 0.5652 | 0.5217 | 0.6087 | 1.0000 |
| 16 | 0.5652 | 0.4783 | 0.3478 | 0.6522 | 0.2174 | 0.4348 | 0.4783 | 0.3913 | 0.5778 | 0.4348 | 0.4783 | 0.4783 | 0.6087 | 0.5652 | 0.5217 |
| 17 | 0.3478 | 0.4348 | 0.3043 | 0.3913 | 0.3043 | 0.3913 | 0.3478 | 0.3478 | 0.5778 | 0.3913 | 0.5652 | 0.6087 | 0.5652 | 0.6087 | 0.5217 |
| 18 | 0.6957 | 0.5652 | 0.5217 | 0.5217 | 0.3478 | 0.5652 | 0.5652 | 0.4783 | 0.6667 | 0.6087 | 0.5652 | 0.6087 | 0.5217 | 0.5217 | 0.4348 |
| 19 | 0.5217 | 0.4348 | 0.3478 | 0.4783 | 0.3913 | 0.4348 | 0.4783 | 0.3913 | 0.5333 | 0.5652 | 0.4348 | 0.4783 | 0.6957 | 0.7391 | 0.6087 |
| 20 | 0.2609 | 0.3478 | 0.2174 | 0.3478 | 0.3478 | 0.2174 | 0.3478 | 0.3913 | 0.5333 | 0.4348 | 0.3478 | 0.4783 | 0.5217 | 0.5217 | 0.4348 |
| 21 | 0.2609 | 0.2609 | 0.2174 | 0.3478 | 0.2174 | 0.3043 | 0.2609 | 0.3913 | 0.4444 | 0.4783 | 0.3478 | 0.4783 | 0.5217 | 0.5217 | 0.3043 |
| 22 | 0.5217 | 0.3043 | 0.4348 | 0.5652 | 0.5217 | 0.4783 | 0.5217 | 0.4348 | 0.3556 | 0.6087 | 0.5217 | 0.5652 | 0.8261 | 0.6087 | 0.4783 |
| 23 | 0.5652 | 0.6087 | 0.5217 | 0.4783 | 0.4783 | 0.3913 | 0.3913 | 0.3913 | 0.5778 | 0.5217 | 0.5652 | 0.6087 | 0.5652 | 0.6522 | 0.6957 |
| 24 | 0.6522 | 0.4783 | 0.5652 | 0.4783 | 0.4348 | 0.5217 | 0.4783 | 0.3043 | 0.5333 | 0.5217 | 0.5217 | 0.5652 | 0.6522 | 0.6522 | 0.6087 |
| 25 | 0.3478 | 0.2174 | 0.2174 | 0.3043 | 0.1739 | 0.2609 | 0.2609 | 0.3478 | 0.2222 | 0.3043 | 0.2609 | 0.3913 | 0.3478 | 0.3043 | 0.3043 |
| 26 | 0.2609 | 0.3043 | 0.3043 | 0.2174 | 0.2174 | 0.2174 | 0.2174 | 0.2174 | 0.2222 | 0.1739 | 0.1739 | 0.3478 | 0.0870 | 0.2174 | 0.2609 |
| 27 | 0.5217 | 0.4348 | 0.3913 | 0.4783 | 0.2609 | 0.3913 | 0.3478 | 0.3478 | 0.4889 | 0.5217 | 0.4348 | 0.5652 | 0.3913 | 0.4348 | 0.2609 |
| 28 | 0.2667 | 0.3111 | 0.2222 | 0.3111 | 0.3111 | 0.1778 | 0.3111 | 0.4444 | 0.2727 | 0.4444 | 0.3111 | 0.3111 | 0.3556 | 0.3556 | 0.2222 |
| 29 | 0.6957 | 0.5217 | 0.5217 | 0.5217 | 0.4783 | 0.5652 | 0.5217 | 0.3478 | 0.5778 | 0.5652 | 0.5652 | 0.6087 | 0.6522 | 0.6522 | 0.5652 |
| 30 | 0.6957 | 0.6087 | 0.6957 | 0.4348 | 0.4783 | 0.5652 | 0.4348 | 0.2609 | 0.4889 | 0.4783 | 0.4348 | 0.6087 | 0.5217 | 0.5652 | 0.5652 |

Appendix 1.The similarity morphological matrix on 30 accessions of salak

| Aksesi | 27 - 18 MWR | 26 - 14 MWR | 12 - 14 Mw-Afn | 12 - 4 Mw-Afn | 23 - 3 SBGP | 24 - 18 SBGP | 18 - 11 SJG | 19 - 27 SL-SJ | 20 - 5 JW-Ph | Glabres-cens | Affinis | Gading Bali | Walicci-ana | 7 - 6 Ph-Jy | 7 - 8 Ph-Jy |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
| 16 | 1.0000 | | | | | | | | | | | | | | |
| 17 | 0.6957 | 1.0000 | | | | | | | | | | | | | |
| 18 | 0.6087 | 0.5652 | 1.0000 | | | | | | | | | | | | |
| 19 | 0.6957 | 0.5217 | 0.5652 | 1.0000 | | | | | | | | | | | |
| 20 | 0.6522 | 0.6522 | 0.4348 | 0.5652 | 1.0000 | | | | | | | | | | |
| 21 | 0.6522 | 0.6522 | 0.4348 | 0.5217 | 0.7826 | 1.0000 | | | | | | | | | |
| 22 | 0.4783 | 0.4783 | 0.4783 | 0.5652 | 0.3913 | 0.3913 | 1.0000 | | | | | | | | |
| 23 | 0.6087 | 0.6087 | 0.6087 | 0.6522 | 0.4348 | 0.3913 | 0.5652 | 1.0000 | | | | | | | |
| 24 | 0.7391 | 0.5652 | 0.5652 | 0.6957 | 0.5652 | 0.5217 | 0.5652 | 0.6957 | 1.0000 | | | | | | |
| 25 | 0.3043 | 0.2609 | 0.2609 | 0.3478 | 0.2174 | 0.2609 | 0.3043 | 0.2609 | 0.2609 | 1.0000 | | | | | |
| 26 | 0.1304 | 0.2174 | 0.2609 | 0.2174 | 0.2174 | 0.2609 | 0.1304 | 0.3043 | 0.1739 | 0.5217 | 1.0000 | | | | |
| 27 | 0.4348 | 0.4348 | 0.6087 | 0.3043 | 0.3913 | 0.5217 | 0.3913 | 0.3478 | 0.3478 | 0.3043 | 0.3043 | 1.0000 | | | |
| 28 | 0.3111 | 0.2222 | 0.2667 | 0.3556 | 0.3111 | 0.2667 | 0.3556 | 0.3556 | 0.2667 | 0.4000 | 0.2222 | 0.3556 | 1.0000 | | |
| 29 | 0.6522 | 0.5217 | 0.6087 | 0.6957 | 0.4783 | 0.4348 | 0.5652 | 0.7391 | 0.8261 | 0.2609 | 0.1739 | 0.4348 | 0.3556 | 1.0000 | |
| 30 | 0.5652 | 0.4783 | 0.5217 | 0.5652 | 0.3913 | 0.3478 | 0.4348 | 0.6522 | 0.8261 | 0.2174 | 0.2174 | 0.3043 | 0.2667 | 0.7826 | 1.0000 |

# Optimalization Flour Composite Nutritiose as Basic Materials Processing for Food Products

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## Abstract

In Indonesian are still many communities that food insecurity and malnutrition. One reason is the consumption pattern and affordability to get a quality product or nutritious food is relatively limited, so it is necessary, efforts to resolve the issue. Therefore, this study was planned to be one of the solutions to overcome the problems of food and nutrition insecurity society. The research is the optimization of the composition of mocaf flour,dregs of tofu, soy pulp, and rice bran in the manufacture of composite flour. The results obtained include: the yield of mocaf flour obtained amounted to 33.55% and has a protein content 2.20%, starch content 75.30%, water content 7.12%, the yield of tofu'sdregs know obtained amounted to 11.2% and has a protein content of 28.62%, starch content 23.42%, water content 9.69 %, the yield of soy pulp obtained at 8.75% and has a protein content 24.18%, strach content 18.80%, moisture content 8.22% and yield of rice bran flour obtained amounted to 37.06% and has a protein content 9.02%, starch content 24.12%, water content 6.69%.

Keywords: optimization, mocaf flour,dregrs of tofu, soy pulp, rice bran

## 1. Introduction

Food and nutrition insecurity in Indonesian society due to food consumption patterns which are not in accordance with the standards of consumption and nutrition of fresh and processed food products consumed, resulting in non-fulfillment of standards Desired food pattern(PPH). Therefore, it is indispensable efforts of various parties to create a food product that is affordable by the lower layers of society and at the same time or a nutritious quality to meet nutritional standards. Nutritional standards is a food product that contains protein, fat, carbohydrates, vitamins, minerals, and functional substances that can nourish the human body. To meet the nutritional needs, then one attempts to do is to create a composite flour as a raw material for processing derivatives of processed food products are relatively inexpensive. How that can be done is to revitalize the waste materials or by-product from the processing industry knows that dregre of tofu, soy pulp, rice bran, and mocaf flour treated as a byproduct of cassava processing industry. So expect the processed products of its food can be affordable and liked by the people.

Dregs of tofu is a byproduct of the tofu industry that they have a relatively high content of nutrients, especially protein content, which is about 7-10%. soy pulp is also a byproduct of the soy industry is also still relatively high protein content 20-28%. Rice bran is a byproduct of rice milling industry has a content of vitamins (Vitamin B1 and B2, as well as other relatively high). While mocafflour is a fermented cassava flour that is a source of carbohydrates (starches) together with a relatively high starch content in cassava, which is about 60- 70%. Seeing the potential of protein and vitamins and

starch that is in the source, then it has the potential to be processed into composite flour nutritional value, which contains proteins, carbohydrate, fats, vitamins, minerals, and substances of functional, so the researchers looked at the need for used as raw material for the manufacture of food processed further.

## 2. Material and Methods

*2.1.Materials*

Materials for the manufacture of composite flour include dregs of tofu, soy pulp, bran and cassava peel. While the materials for the purposes of chemical analysis includes reagents for the analysis of protein, carbohydrate, fat, and fiber.Tools for manufacture of composite flour covering dryer, flour miller, slicer, screen, mixer, and tools for analysis.

*2.2.Method*

The study is expected to produce composite flour nutritious as raw materials for advanced processing of derivative products. The research method includes a preliminary stage and the main stage of research. Preliminary phase of the research is to analyze the chemical dregs of tofu, soy pulp, and rice bran, and make processing flour of tofu's dregs,soy pulp flour, and rice bran flour and mocaf flour. The main phase of the study is to formulate the composition of flour of tofu's dregs ($t_1$), soy pulp flour ($t_2$), rice bran flour ($t_3$), and mocaf flour ($t_4$) with the formulation as follows:

- f1 is $t_1 : t_2 : t_3 : t_4 = 1 : 3 : 1 : 5$
- f2 is $t_1 : t_2 : t_3 : t_4 = 2 : 2 : 1 : 5$
- f3 is $t_1 : t_2 : t_3 : t_4 = 3 : 1 : 1 : 5$
- f4 is $t_1 : t_2 : t_3 : t_4 = 3 : 5 : 2 : 10$
- f5 is $t_1 : t_2 : t_3 : t_4 = 5 : 3 : 2 : 10$

The experimental design will be used in this study is a randomized block design with five treatments and five replications, so therefore there will be a 25 experiment. To prove the effect of the formulation of all responses observed variables, the analysis data, using the experimental design as follows:

$Yij = \mu + \tau i + \varepsilon ij$ [1]

Based on the above experimental design can be made analysis of variance (ANOVA), to obtain conclusions about the effect of treatment formulations. The response to research that analyzed the composite flour of various formulations (the analysis of protein content, starch content, fat content, fiber content, moisture content and ash content).

## 3. Result and Discussion

*3.1.*Result

Raw materials analysis was conducted to determine the chemical composition of cassava peel, dregs of tofu, soy pulp, and rice bran are used as raw material in the manufacture of composite flour. The results of chemical analysis of raw materials can be seen in Table 1.

Flour analysis was conducted to determine the chemical composition mocaf flour, flour of tofu's dregs, soy pulp flour, and rice bran flour are used as the main raw material in the manufacture of composite flour. The results of chemical analysis mocaf flour, flour of tofu's dregs, soy pulp flour, and rice bran flour can be seen in Table 2.

Table1. Analysis of Chemical Raw Materials

| Type of Material | Results of Analysis | | | | | |
|---|---|---|---|---|---|---|
| | Protein Content (%) | Strach Content (%) | Fat Content (%) | Fiber Content (%) | Water Content (%) | Ash Content (%) |
| Cassava peel | 1.14 | 44.80 | 0.38 | 5.91 | 45.12 | 1 |
| Dregs of tofu | 8.40 | 9.62 | 9.71 | 10.66 | 59.08 | 2.93 |
| Soy pulp | 10.88 | 9.75 | 7.80 | 16.10 | 45.61 | 2 |
| Rice bran | 8.72 | 23.42 | 4.51 | 42.94 | 14.69 | 3.50 |

Table 2.Results of Chemical Analysis Mocaf Flour, Flour of Tofu's Dregs, Soy Pulp Flour, and Rice Bran Flour

| Type of flour | Results of Analysis | | | | | |
|---|---|---|---|---|---|---|
| | Protein Content (%) | Strach Content (%) | Fat Content (%) | Fiber Content (%) | Water Content (%) | Ash Content (%) |
| Mocaf flour | 2.20 | 75.30 | 0.68 | 10.82 | 7.12 | 2.50 |
| Flour of Tofu's Dregs | 28.62 | 23.42 | 14.71 | 16.96 | 9.69 | 3.50 |
| Soy Pulp Flour | 24.18 | 18.45 | 16.80 | 23.12 | 8.22 | 2.95 |
| Rice Bran Flour | 9.02 | 24.12 | 4.80 | 49.34 | 6.69 | 2.80 |

Table 3. Results of Chemical Analysis Flour Composites

| Chemical Characteristics | Formula I | II | III | IV | V |
|---|---|---|---|---|---|
| Protein Content (%) | 12,94 | 13,00 | 13,21 | 13,79 | 13,73 |
| Fat Content (%) | 7,33 | 7,12 | 9,97 | 10,29 | 7,02 |
| Strach Content (%) | 48,14 | 48,84 | 47,04 | 45,49 | 49,18 |
| Fiber Content (%) | 18,98 | 18,36 | 17,75 | 18,67 | 18,05 |
| Water Content (%) | 7,36 | 7,82 | 7,98 | 7,74 | 7,88 |
| Ash Content (%) | 2,76 | 2,82 | 2,88 | 2,79 | 2,85 |

Tabel 2 shows that the difference in the type of flour affect the value of the protein content, strach content, fat content, fiber content,water content, ang ash content. All type of flour are used as a flour substitute or composite flour for the manufacture of products, so it can produce a product that is more diverse and can reduce the use of wheat flour.

In the process of making mocaf flour obtained mocaf flour weighing 2096 grams from 6247 grams of cassava peel, so the yield of mocaf flour obtained at 33.55%. In the process of making flour of tofu's dregs obtained 280 grams of flour with a weight of 2,500 g pulp, so that the yield of starch Dregs of tofu obtained amounted to 11.2%. In the process of making soy pulp flour obtained 175 grams of flour weighing 2,000 grams of soy pulp, so that the yield of soy pulp flour obtained at 8.75%. In the process of making rice bran flour obtained bran flour weighing 5000 grams from 1853 grams of rice bran, bran flour thus obtained yield of 37.06%.

a. Protein levels

Based on the ANOVA can be seen there is a noticeable effect on the level of 5% protein contentof composite flour

Table 4. Protein Content(%) of Composite Flour with several formulation

| Treatment | Protein Content |
|---|---|
| f1 | 12.94[a] |
| f2 | 13.00[b] |
| f3 | 13.21[c] |
| f5 | 13.73[d] |
| f4 | 13.79[e] |

Information:The same letter in the column showed no significant difference in the level of 5%

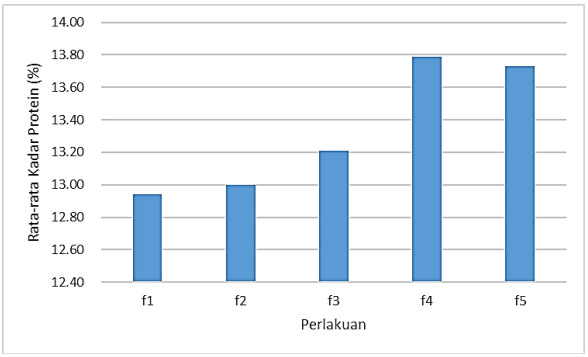


Figure 1. Influence Formulation on Protein Content of Composite Flour (%)

b. Fat level

Based on the ANOVA can be seen there is a noticeable effect on the level of 5%fat contentof composite flour

Table 5. Fat Content(%)of Composite Flour with several formulation

| Treatment | Fat Content |
|---|---|
| f5 | 7.02[a] |
| f2 | 7.12[b] |
| f1 | 7.33[c] |
| f3 | 9.97[d] |
| f4 | 10.29[e] |

Information:The same letter in the column showed no significant difference in the level of 5%

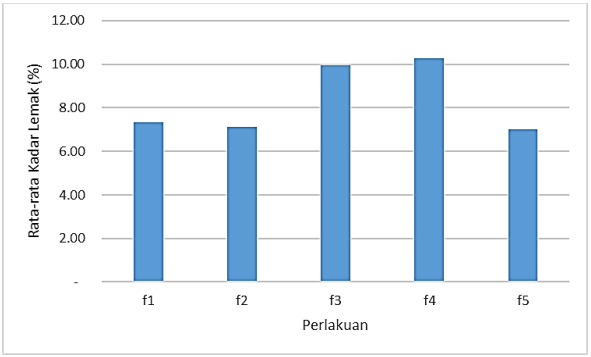


Figure 2. Influence Formulation on Fat Content of Composite Flour (%)

c. Starch Content

Based on the ANOVA can be seen there is a noticeable effect on the level of 5%strach contentof composite flour

Table 6. Starch Content(%) of Composite Flour with several formulation

| Treatment | Starch Content |
|---|---|
| f4 | 45.49[a] |
| f3 | 47.04[b] |
| f1 | 48.14[c] |
| f2 | 48.84[d] |
| f5 | 49.18[e] |

Information:The same letter in the column showed no significant difference in the level of 5%

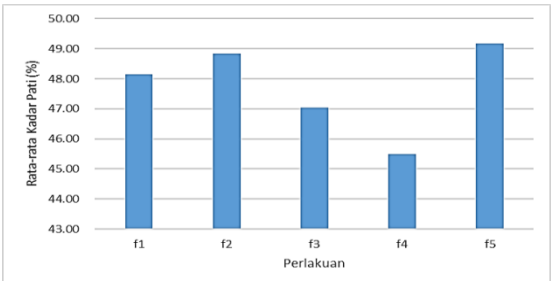


Figure 3. Influence Formulation on Strach Content of Composite Flour (%)

d. Fiber Content

Based on the ANOVA can be seen there is a noticeable effect on the level of 5%fiber contentof composite flour

Table 7. Fiber Content(%) of Composite Flour with several formulation

| Treatment | Fiber Content |
|---|---|
| f3 | 17.75[a] |
| f5 | 18.05[b] |
| f2 | 18.36[c] |
| f4 | 18.67[d] |
| f1 | 18.98[e] |

Information:The same letter in the column showed no significant difference in the level of 5%

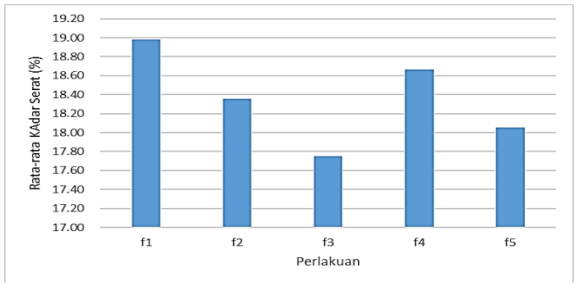


Figure 4. Influence Formulation on Fiber Content of Composite Flour (%)

d. Water Content

Based on the ANOVA can be seen there is a noticeable effect on the level of 5% water content of composite flour.

Table 8. Water Content(%) of Composite Flour with several formulation

| Treatment | Water Content |
|---|---|
| f1 | 7.36[a] |
| f4 | 7.74[b] |
| f2 | 7.82[c] |
| f5 | 7.88[d] |
| f3 | 7.98[e] |

Information:The same letter in the column showed no significant difference in the level of 5%

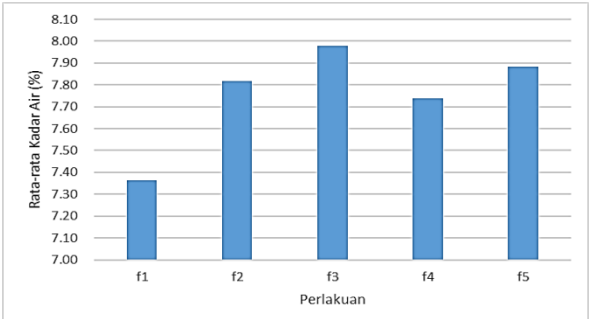


Figure 5. Influence Formulation on Water Content of Composite Flour (%)

f. Ash Content

Based on the ANOVA can be seen there is a noticeable effect on the level of 5%ash contentof composite flour.

Table 9. Ash Content(%) of Composite Flour with several formulation

| Treatment | Ash Content |
|---|---|
| f4 | 2.76[a] |
| f1 | 2.79[ab] |

| | |
|---|---|
| f2 | $2.82^{bc}$ |
| f5 | $2.85^{cd}$ |
| f3 | $2.88^{d}$ |

Information:The same letter in the column showed no significant difference in the level of 5%

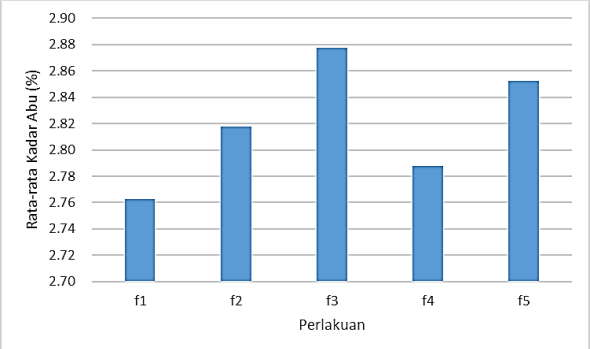


Figure 6. Influence Formulation on Ash Content of Composite Flour (%)

*3.2.*Discussion

Evaporation of water from the material to the hot dry air that occurs because of differences between the relative humidity of the dry air at the surface of the dried material causing the driving force or a pressure difference of water vapor in the water vapor pressure materials with dry air. The situation is causing the evaporation of moisture from the material to air dry. Drying of a material by blowing hot dry air that resulted in the evaporation rate of water from the dried material will increase [2]. Events evaporation of water from the dried material begins with the depletion layer of air barrier between the surface of the material dried with air environment due to the hot dry air blowing. Blowing hot dry air that is given continuously during drying results in an increase in the vapor pressures difference dean material surface with the water vapor pressure in the dry air so that water on the surface of the material will evaporate. The difference in concentration of water causes water in the material will diffuse into the surface of the material and the evaporation of water. This occurs until equilibrium is achieved between the water content in the material with water content in the air is dry.

Protein is a nutrient that is essential for the body because these substances in addition to functioning as a fuel in the body also serves as a builder substance and regulators [3]. Based on the results obtained by analysis of protein content in mocaf flour is 2.20% while according to Codex Stan 176-1989 expressed protein content in mocaf flour from meat casava maximum of 1.0% [4]. The protein content is slightly higher than mocaf flour made of meat cassava because of a protein in 100 grams of cassava peel is 8.11 g, while in 100 g of meat cassava protein content of only 1g so that we can conclude the protein content of cassava peel is higher than the protein content of meat cassava, supposedly this is very influential on the protein content of the flour produced mocaf [5].

The carbohydrate content of fresh cassava peel is 4.55%, making it possible to use as an energy source for microorganisms in the fermentation process. Besides cassava peel also contains tanins, enzymes peroxide, glycated, calcium oxalate, fiber, and HCN [6].The content of HCN in cassava peel can be reduced through several treatments include soaking, boiling and fermentation. The fermentation process can reduce the content of HCN and increasing the energy content, protein, crude fiber, as well as improve the digestibility of low-quality food ingredients [7]. The microbes used in the fermentation process can produce enzymes that will degrade complex compounds into simpler and synthesize a protein that is a protein enrichment process material (protein enrichment). This microbial protein commonly called Single Cell Protein [8].

Based on the results obtained by analysis of protein content in dregs of tofu amounted to 28.62%. Protein content dregs of tofuis higher due to the processing of dregs of tofu is not too much squeezed while still wet, when the fresh dregs of tofu obtained directly carried steaming to reduce the water content

and drying so that the protein content is high and quite good.

Based on the results obtained by analysis of protein content in soy pulp flour amounted to 24.18%. The result is not too much different because the process of making soy pulp carried out by the same process by soaking in water at room temperature not hot water that can damage proteins. Immersion aims to eliminate the high levels of NaCl on the lees. Soy pulp has a relatively high nutrient content, especially protein, because in the process of making soy sauce only a small percentage of soy protein and soluble utilized in soy sauce, while the remaining dregs left in soy sauce [9].

Based on analysis of the protein content of the rice bran flour is at 9.02%. States that proteins are very sensitive to heat and will change the chemical structure (denaturation) as a result of warming [10]. States high heat will cause degradation of the protein molecules [11]. The degradation products produced many protein derivative which is soluble in water.

Based on the analysis of water content in mocafflourat 7.12%, flour of tofu's dregsat 9.69%, soy pulp flour at 8:22%, and 6.69% at rice bran flour. In research Codex Stan 176-1989 stated maximum water content in flour mocaf is 13% so that it can be concluded mocaffrom cassava peel within limits.

Ash is one component in foodstuffs. This component consists of minerals such as potassium, phosphorus, sodium, magnesium, calcium, iron, manganese, and copper [12]. Minerals is one of the essential nutrients needed by the body in small amounts. Based on analysis of the ash content of 1% mocaf flour, flour of tofu's dregs3.50%, and the soy pulp flour amounted to 2.95%, while the rice bran flour amounted to 2.80%. The relatively low ash content allegedly because the mineral nutrient content of non-starch flour are high enough, resulting in the content of ash in the flour in this study is low. This is consistent with the statement of Nabil that the lower non-mineral components contained in the materials will further lower the percent of ash in the material [13]. The ash content depending on the type of material, means of ashing, the time and temperature used during the drying.

Based on analysis of the fiber content in mocaf flour amounted to 10.82%, 16.96% on flour of tofu's dregs, soy pulp 23.12%, and rice bran flour49.34%. Results fiber content in flour is relatively higher than the raw material, and this is one of the components contained in the material which led to reduced water levels rising flour fiber.

Drying have an influence on the nutrients, because the heat can cause degradation in these nutrients especially the provision of heat. Damage nutrients in dried foodstuffs closely linked to temperature and drying time. The increasing drying time and temperature will increase in losses of nutrients.

Based on the analysis of variance (ANOVA) with a model in one direction (one way) against the five formulas ($f_1$, $f_2$, $f_3$, $f_4$ and $f_5$) mixing the mocaf flour, flour of tofu's dregsflour, soy pulp flour, and rice bran flour shows the difference significantly for the response levels of protein, starch, fat, fiber, water, and ash at the 5% significance level.The real difference of the components caused by the composition of the raw starch containing macro nutrients are not the same, so that the resulting composite flour which has a different composition for protein, fat, starch, fiber, water and ash. The basic ingredients of soy pulp and dregs of tofu provide a response to the protein and fat content is relatively high, while the base material rice bran responded fiber and ash are relatively higher than the source material other basic, basic materials mocaf respond starch content is relatively higher than on sources other basic materials.

Based on the survey results revealed that each portion contains the basic ingredients have nutritional components that

are not the same and each has specific characteristics. Mocaf flour is oriented as a source of carbohydrate (starch), while other basic materials are relatively less starch content. The basic ingredients of soy pulp and dregs of tofu oriented as a source of protein and fat, while mocaf flour and rice bran protein content is low, but on the other rice bran is rich in fiber. So therefore the results of this study are targeted to create composite flour of high nutritional value, protein, fat, starch, water and fiber are proportional, so expect the composite flour can be used as a source of raw materials to the processing of derivative food products nutritional value high to meet the nutritional needs for consumers society in general.

The response to the ash content, $f_1$ to $f_4$ treatment showed no significant difference, $f_1$ to $f_2$ treatment showed no significant difference, $f_2$ to $f_5$ showed no significant difference, and with $f_3$ to $f_5$ showed no significant difference. However, ash and water is not one of the considerations to determine the selection of the use of composite flour as a raw material in the processing of food products derived further, but only used as a reference characteristic

## Conclusion

Based on preliminary research results can be summed content of the raw materials can be used as the manufacture of composite flour that can be used in the manufacture of food products. The yield of mocaf flour obtained at 33.55%, the yield of Flour of Tofu's Dregs obtained for 11.2%, the yield of soy pulp obtained at 8.75% and the yield of rice bran flour obtained at37.06%.

The use of composite flour for processing raw materials derived food products can then be considered as follows:

a. If considering a composite flour as raw materials for food products derived by considering the levels of protein and fat, it can be used formula $f_4$, the composite flour containing 13.79% protein and fat at 10.29%.
b. If considering a composite flour as raw materials for food products derived by considering the starch content, it can be used formula $f_5$, the composite flour containing starch amounted to 49.18%.
c. If considering a composite flour as raw materials for food products derived by considering the fiber content, it can be used formula $f_1$, ie composite flour contains fiber by 18.98%.

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## References

[1] Gasperz, Vincent, 1995. *Teknik Analisis dalam Penelitian Percobaan*, Bandung: Tarsito, .
[2] Desrosier, Norman W., *Teknologi Pengawetan Pangan. Edisi Pertama*, Jakarta: Penerbit Universitas Indonesia, 1988.M.
[3] Winarno, F. G., *Kimia Pangan dan Gizi*, Jakarta: PT. GramediaPustakaUtama, 1992.
[4] Subagio, A.,*Industrialisasi Modified Cassava Flour (Mocaf) Sebagai Bahan Baku Industri Pangan Untuk Menunjang Diversifikasi Pangan Pokok Nasional*. Jember: Fakultas Teknologi Pertanian, Universitas Jember, 2007.
[5] Mahmud, *Tabel Komposisi Pangan Indonesia*, Jakarta: PT Elex Media Komputindo, 2009.
[6] Arifin. *Kandungan Gizi pada Ubi Kayu*. Jurnal Ilmu-ilmu Peternakan. IX (2): 90-110, 2005.
[7] Turyoni, D., *Pembuatan Dodol Tape Kulit Singkong (Cassava) Teknologi Jasa dan Produksi*, Semarang: Universitas Negeri Semarang, 2005.
[8] Darmawan, *Pengaruh Kulit Umbi Ketela Pohon Fermentasi terhadap Tampilan Kambing Kacang Janta*, Jurnal Ilmiah Ilmu-Ilmu Peternakan, 9 (2) : 115-122, 2006.
[9] Mulyokusumo, S,E., *Kecap*, Bandung:Terate, 1974.
[10] Damayanthi E, Tjing LT &Arbianto L, *Rice Bran*, Depok: Penebar Swadaya, 2007.

[11] Winarno, F.G., *Enzim Pangan*, Jakarta:Gramedia Pustaka Utama, 1995.

[12] Nabil, M., *Pemanfaatan Limbah Tulang Ikan Tuna (Thunnus sp.) Sebagai Sumber Kalsium Dengan Metode Hidrolisis Protein*, Program Studi Teknologi Hasil Perikanan, IPB. Bogor. 2005.

[13] Sudarmadji, S., B. Haryono, Suhardi, *Prosedur Analisa untuk Bahan Makanan dan Pertanian*, Yogyakarta: Penerbit Liberty, 1997.

# $KNO_3$ Concentration and Soaking Time Effect on Breaking Seed Dormancy and Seed Growth of Sour-Sop (*Annona muricata* L.)

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## Abstract

This study has been implemented in Jalan Delitua, Deli Serdang regency began in February and was completed in May 2015. This study used seed sour-sop (*Anonna muricata* L.) and method used a randomized block design factorial with two factors namely $KNO_3$ concentration(K) with 4 levels: $K_0$=0%, $K_1$=0,1%, $K_2$=0,3%, $K_3$=0,5% and Soaking time with 3 levels:$W_1$=24 hours, $W_2$=48 hours and $W_3$=72 hours. There were repeated three times. The parameters observed are the maximum potential growth, germination, vigorindex, speed of growth, simultanenously growth, height of seed, number of leaves, leaf area, fresh weight and dry weight of plants. The results showed that the concentration of$KNO_3$ 0,5% and soaking time of 72 hours was able to breaking seed dormancy faster than other treatments. The concentration of$KNO_3$ 0,5% affect the plant growth with the highest seedling height was 20.75 cm, the highest number of leaves was 11.81 strands and widest of leaf area was 18.22$cm^2$.The soaking time of 72 hours affect the plant growth with the highest seedling height was 18.65 cm, the highest number of leaves was 10.44 strands and the widest of leaf area was 16.90 $cm^2$.

Keywords: $KNO_3$ concentration, soaking time, sour-sop, dormancy, seed

## 1. Introduction

*A. muricata* is known as sour-sop (English), graviola (Portuguese), guanábana (Latin American Spanish) and other local indigenous names [1]. Sour-sop is rising popularity of fruit plants into medicinal plants. The many benefits of sour-sop make people start turning to the sour-sop as an alternative to conventional prevention and medication [2].Bioactive compounds derived from plants sour-sop Annonaceousacetogenin, have long been investigated and shown to be anti-cancer, but it also is antiparasitic, insecticides, anti-worm, antibacterial, and antiviral [3].Phytochemical content of this plant is acetogenin, alkaloids, quinoline, isokuinolin, tannins, coumarin, prosianidin, flavonoids, amyl caproate [4].Sour-sop fruit has a high content of polyphenolic compoundsand contains vitamin C [5] [6].Besides the vitamin C content also affects the activity antioksidan [7].In sour-sop cultivation, farmers in the field a lot of obstacles.one of the factors that influence the failure of plant propagation generative via seeds is low ability of seeds to germinate [8].Low ability of seeds to germinate can be caused by several factors: the level of maturity of seeds, seed size, dormancy, germination inhibitors, water, temperature, oxygen, and light [9].Sour-sop seeds are sown slow because of the level of violence induced germination seeds skin, the harder seed coat, the time required to inducegermination is also getting longer [10].

Potassium nitrate ($KNO_3$) is most widely used chemical for promoting seed germination of many species [11]. The concentration used for various types of seeds certainly not the same, depending on the

characteristics of the seeds used [6].International Seed Testing Association (ISTA) recommended the use of $KNO_3$ with a concentration of 0.1 to 0.2% [12]. $KNO_3$ used as a germination promoter in the majority of seed germination testing. $KNO_3$ concentration of 0.2%, 0.3%, 0.4% strongly affect the texture of hard surface oil palm seeds become more flexible when compared with controls.The advantages of $KNO_3$ that can help the formation of flowers and fruit, increases plant resistance to disease increase plant resistance to drought [13**].**

## 2. Material and Methods

This research was conducted in Februariand was completed in May 2015, at Jalan Deli Tua, Deli Serdang regency, Province of Sumatera Utara. Materials used in this research is the seed sour-sop, a solution of potassium nitrate ($KNO_3$), NPK fertilizer, water, polybag, and others.The method used arandomized block design factorial with two factors namely $KNO_3$ concentration(K) with 4 levels:$K_0$=0%, $K_1$=0,1%, $K_2$=0,3%, and $K_3$=0,5% and Soaking time with 3levels:$W_1$=24 hours, $W_2$=48 hours and $W_3$=72 hours. There were repeated three times.

The collected data were analyzed statistically with ANOVA and differences between treatment means were compared using the DMRT at probability level of 0.05. Seed treatment by soaking in accordance with the concentration and length of time of soaking sowing using germination trays that have been filled with soil topsoil mixed with sand in the ratio 1: 1 in accordance with the seed treatment.

Seeds are soaked as much as 30 seeds of each treatment were separated into 5 seeds every 1 petri dish.The parameters were observed: *Seed Germination* namely the maximum potential growth, germination, vigorindex, speed of growth, simultanenously growth, and *The Growth*namely height of seed, number of leaves, leaf area, fresh weightanddry weight of plants.

## 3. Results and Discussion

### *3.1. Seed Germination*

Based on the research that $KNO_3$ role in seed dormancy breaking sour-sop, this can be seen from the percentage of potential growth, germination, vigor index, speed of growth and simultanenously growth (Table 1.).At a concentration of 0.5% ($K_3$) sour-sop seed dormancy breaking faster than with a concentration of 0.1% ($K_1$), 0.3% ($K_2$) and without treatment ($K_0$) with germination time ranging from 14 days - 32 days.This is caused by the presence of $KNO_3$ treatment that can improve the speed of sprouted seeds. Pretreatment with a solution of $KNO_3$role stimulate germination in almost all types of seeds [14]. $KNO_3$, or Potassium Nitrate is one of the germination stimulants are frequently used and have a strong influence on the percentage of germination and seed vigor [15].One of the factors that determine seed quality is the level of maturity. Seed vigor reached a maximum at the time of physiological maturity. Seeds were harvested after reaching physiological maturity had relatively higher vigor so that it will produce plants more vigor and have a longer life. The seeds which have physiological maturity will have perfect food reserves that can support the growth of sprouts [16]. The level of maturity of the seeds can be characterized from maturity level. Seed quality reaches its maximum at the time of physiological maturity is characterized by dry weight and vigor maximum [17].

Hard seed coat that is impermeable to water and air, thus blocking the process of seed germination. $KNO_3$ with a certain concentration can stimulate growth [18]. Potassium nitrate ($KNO_3$) is an inorganic salt which is specifically referred to as chemicals that will greatly affect dormancy breaking treatments [19].

Table 1. Seed Germination Sour-sopat Age 14-32 Days

| Treatment | Maksimum of Potentialgrowth | Germination | VigorIndex | Speed of Growth | Simultanenouslygrowth |
|---|---|---|---|---|---|
| | | | (%) | | |
| $K_0W_1$ | 50,92 | 43,33 | 13,33 | 2,05 | 44,43 |
| $K_0W_2$ | 50,00 | 43,33 | 16,57 | 3,25 | 55,23 |
| $K_0W_3$ | 52,38 | 46,67 | 20,00 | 35,26 | 66,67 |
| $K_1W_1$ | 51,43 | 53,33 | 23,33 | 36,10 | 77,77 |
| $K_1W_2$ | 52,86 | 53,33 | 23,33 | 38,50 | 77,77 |
| $K_1W_3$ | 52,86 | 53,33 | 23,33 | 39,00 | 77,77 |
| $K_2W_1$ | 51,90 | 53,33 | 23,33 | 39,01 | 77,77 |
| $K_2W_2$ | 50,47 | 63,33 | 23,33 | 39,00 | 77,77 |
| $K_2W_3$ | 51,90 | 66,67 | 26,67 | 40,00 | 89,00 |
| $K_3W_1$ | 51,90 | 70,00 | 26,67 | 40,25 | 89,00 |
| $K_3W_2$ | 53,04 | 80,00 | 30,00 | 40,33 | 100,0 |
| $K_3W_3$ | 53,54 | 83,33 | 30,00 | 40,85 | 100,0 |

Tabel 2. Effect Concentration $KNO_3$ and Soaking Time on Seedling Growth of Sour- sopat 8 Weeks After Planting(WAP)

| Treatments | Parameters | | | | | | |
|---|---|---|---|---|---|---|---|
| | Heigh of Seed (cm) | Number of leaves (helai) | Leaf Area ($cm^2$) | Fresh weight up (g) | Fresh weight down (g) | Dry weight up (g) | Dry weight down (g) |
| | | | Consentration $KNO_3$ | | | | |
| $K_0$ | 16,30c | 8,56d | 14,60d | 4,64 | 1,40 | 1,07 | 0,39 |
| $K_1$ | 17,62bc | 9,52c | 15,83c | 4,85 | 1,39 | 1,10 | 0,32 |
| $K_2$ | 18,45b | 10,37b | 17,14b | 4,63 | 1,41 | 1,10 | 0,28 |
| $K_3$ | 20,75a | 11,81a | 18,22a | 5,09 | 1,49 | 1,23 | 0,32 |
| | | | Soaking Time | | | | |
| $W_1$ | 17,85b | 9,67bc | 16,01bc | 4,53 | 1,36 | 1,10 | 0,33 |
| $W_2$ | 18,35ab | 10,08b | 16,44b | 4,93 | 1,40 | 1,17 | 0,32 |
| $W_3$ | 18,65a | 10,44a | 16,90a | 4,95 | 1,51 | 1,12 | 0,34 |

Note :Value in the same column followed by the same letters are not significantly different at the p<0,05 level according to DMRT

The granting of $KNO_3$ concentration of 0.2%, 0.3%, 0.4% strongly affect the texture surface hardness of hard seeds become more flexible when compared with controls. The concentration used for various types of seeds certainly not the same, depending on the characteristics of the seeds concerned. External and internal factors seed becomes a very important factor in the growth process [12].

$KNO_3$ concentration sour-sop effect on seedling growth, seedling height, number of leaves and leaf area at the age of 8 Weeks After Planting (WAP), with high growth are on the highest seed treatment $K_3$ (concentration of 0.5%) with a height of 20.75 cm. In the treatment of $K_2$with18.45 cm high.In the treatment of $K_1$ with 17.62 cm high.In the treatment of $K_0$ (without treatment) growth of soursop seedlings tend to be lower and slower with 16.30 cm high. Effect of KNO3 incurred is determined by a small large concentration [14].At the highest growth in the number of leaves that are in treatment $K_3$as much as 11.81 strands. In the treatment of $K_2$ number of leaves as much as 10.37 strands. In the treatment of $K_3$ number of leaves as much as 9.52 strands. In the treatment of $K_0$ (without treatment) the number of leaves less compared to other treatments as much as 8.56 strands. On the growth of leaf area, $K_3$ treatment is the largest being in an area of 18.22 $cm^2$, compared to $K_2$ area of 17.14 $cm^2$. In the treatment of $K_1$ area of 15.83 cm2 and $K_0$ (without treatment) covering an area of 14.60 $cm^2$.$KNO_3$ serves to increase the activity of growth hormone on the seed[14].Many nitrogen-containing compounds, promote dormancy release and seed germination in many species [21]. $KNO_3$ raises the ambient oxygen levels by making less oxygen available for citric acid cycle [22]. Functioning of nirates in seed germination through reduction to ammonium ions as occurs in the nutrition of plants [21].

Based on breaking seed dormancy soaking time best soursop at the time of soaking of 72 hours.The treatment of soaking in water is used to wash the substances that inhibit germination and may soften the seed coat. The longer the seeds soaked the longer the seeds absorb $KNO_3$ solution, so that seeds become softer. From the research that has been done soaking time significantly affected plant height age 8 WAP. In observation of the number of leaves and leaf area shows the real effect of soaking time at age 8 WAP. At the highest seed soaking time high of 18.65 cm, the highest number of leaves 11.81 and the widest of leaf area 16.90 $cm^2$.

Pre treatment with a solution of $KNO_3$role stimulate germination in almost all types of seeds. The treatment of soaking in a solution of $KNO_3$ reportedly can also activate cell metabolism and speed up germination.

The length of time soaking in a chemical solution is a strong acid such as $KNO_3$ with a dense concentration of the seed makes the skin softer so it can be passed by the water with ease.

Seed soaking time can be determined based on the texture of the seeds themselves. Several types of seeds are sometimes treated submersion in water, which facilitates the absorption of water by the seed. The treatment of soaking in water is used to wash the substances that inhibit germination and may soften the seed coat. Soaking can stimulate faster absorption. Soaking is a procedure that is very slow to cope with the physical dormancy [23]. At the time of soaking effect on the increase in moisture content of the seeds where the soaking time 72 hours had the highest water content than the soaking time 24 and 48 hours. This shows that the treatment of seeds capable of giving real effect to the addition of seed moisture content during the treatment. The variety difference as one of the reasons for differential water uptake by seeds. The most seeds swollen in water and sown in moistenvironment, germinate faster than untreated seeds [24].

The treatment soaking in a solution of $KNO_3$ reportedly can also activate cell

metabolism and speed up germination [14]. Dormancy can also be overcome by the use of chemicals in the stimulation of germination of seeds, for example: $KNO_3$ instead of light function and temperature as well as to expedite the receipt of the seed will be oxygen [25]. High water levels would trigger a more rapid respiration. It is because the respiration rate will be increased after the start of absorption of water by the seeds and show the ingress of oxygen into the seed has been rapid. $KNO_3$ has also been proven effective breaking dormancy of some seeds, such as rice and palm sugar [14]. $KNO_3$ treatment is economic and easily applicable by nursery workers and poor farmes in developing mass planting stock, over costly plant growth regulators and associated technicalities [26].

## Conclusion

1. The concentration of $KNO_3$ 0.5% and soaking time of 72 hours was able to breaking seed dormancy faster than other treatments.
2. The concentration of $KNO_3$ 0.5% affectthe growth with the highest seedling height was 20.75 cm, the highest number of leaves was 11.81 strands andwidestof leaf area was 18.22 $cm^2$.
3. The soaking time of 72 hours affect the growth with the highest seedling height was 18.65 cm, the highest number of leaveswas10.44 strands and the widest of leaf area was16.90 $cm^2$.

## References


[1] A.C. Pinto, Q. de, M.C.R. Cordeiro, S.R.M. De Andrade, F.R. Ferreira, H.A. Filgueiras, C. de, R.E. Alves, D.I. Kinpara, *Annona muricata*, ,in: J.T. Williams (Ed.), Annona Species, Taxonomy and Botany Inter-national Centre Underutilised Crops, Southampton, UK: University of Southampton, pp. 3–16, 2005.

[2] Adjie, S, Sour-sop the Enormity of Assassinate Diseases Jakarta: Pustaka Bunda, 2011.

[3] Taylor L, 2012. *Annona muricata*. Herbal Secret of the Rainforest: The Healing Power of over 50 Medicinal Plants you Should Know About.. http://www.raintree.com/graviola.htm#.

[4] Lim T.K, Annona muricata. In: Edible Medicine and Non Medicine,Fruits, Lim, T.K. (Edt). Dondhrect Holdberg London New

[5] Radi J,Soursop, Cultivation and Utilization. Yogyakarta:Kanisius,1997.

[6] Bora PS, Holschuh HJ, dan Vasconcelos MAS. Characterization of Polyphenol Oxidase of Sour-sop (*Annona muricata* L.) Fruit and Comparative Studyof its Inhibition in Enzyme Extract and in Pulp. Cienciay TecnologiaAlimentaria, 4:267-273, 2004.

[7] Haciseuki A. An overview of Ascorbic Acid Biochemistry. J Fac Pharm Ankara. 38:233-255, 2009.

[8] Agromedia. Key to Success Increase Plant. Jakarta: Agromedia Pustaka, 2007.

[9] Sutopo, L,Seed Technology.. Jakarta: Raja Grafindo Persada, 2002.

[10] Ashari, S,.Horticultural Aspects of Aquaculture. Jakarta: University Indonesia, 1995.

[11] Agrawal, P.K., Dadlani, M., Techniques in Seed Science and Technology. SecondNasri F., et al. Trakia Journal of Sciences,Vol.12, No.1, 2014 45 Edition. SouthAsian Publishers New Delhi International Book Company Absecon Highlands, pp.109-113, 1995.

[12] ISTA. International Rules for SeedsTesting, Seeds Science and Technology,Suplement. International Seeds Testing Assosiation Zurich, 1996, Vol 24.

[13] Fahmi, I.Z, Fracture Treatment Study of Seed Dormancy with Mechanical and Chemical Scarification. Great Hall Plantation Seed and Plant Protection. Surabaya, 2013.

[14] Faustina, E., Yudono, P., and Rabaniyah, R, Method Influence Release of Aril and $KNO_3$Concentration AgainstBreaking Dormancy Papaya Seed. JournalVegetalika,Vol 1. No. 1, 2012.

[15] Schmidt L. Guidelines for Handling Seed Plants Tropical and Subtropical Forests.Jakarta: Directorate General of Land Rehabilitation and Social Forestry, 2000.

[16] Copeland,L.O., and Mc Donald, M.B, Principle of Seed Science and Technology. 4thedition. NewYork : Chapman and Hall, 2001.

[17] Ilyas, S. 2012. Seed Science and Technology: Theory and Research Results. IPB Press. Bogor. p.138. 2012.

[18] Viarini, S, A. $KNO_3$ treatment and Incubation Temperature Effect on Oil Palm SeedDormancy Fracture (*Elaeisguineensis* Jacq var Tenera).Thesis, Fakulty of Agriculture. University Gadjah Mada, 2007.

[19] Danoesastro, H. Plant Growth Regulator in Agriculture. Yogyakarta: Faculty of Agriculture UGM. p. 115, 1993.

[20] John E, Paul ER,TheBiology of Horticulture. An introductory Textbook. Great Britain; John Willey and Sons, W.C,p. 348, 1993.
[21] Bethke, P.C., Libourel, I.G., Jones, R.L., Nitric Oxide in Seed Dormancy and Germination. In: Bradford K J, Nonogaki H (eds), Seed Development, Dormancy and Germination, Blackwell Publishing, Oxford, pp, 153–175, 2007.
[22] Bewley, J. D., and Black, M. Physiology and Biochemistry of Seeds in Relation to Germination. Volume 2: Viability, Dormancy and Environmental Control. Berlin: Springer, 1983
[23] Silomba, S, D, A. Effect of Soaking and Heating Lama against Palm Oil Seed Viability Palm Oil. Thesis. Institute Pertanian Bogor. 2006.
[24] Copeland LO, Principles of Seed Science and Technology, Burges Pub. Co.U.S.A. pp.55-200. 1976
[25] Kartasapoetra, A.G., Seed Technology (Seed Treatment and Practical Guidance),Jakarta: Rineka Cipta. p.188, 2003.
[26] F. Nasri., M. Koshesh Saba, N. Ghaderi, A. Akbar Mozafari, T. Javad. ImprovingGermination And Dormancy Breaking In Alstromeria Ligtu Hybrid Seeds Department of Horticulture Science,Agricultural Faculty, University of Kurdistan, Sanandaj, Iran. Trakia Journal of Sciences, Vol. 12, No. 1, 2014.

# Effect of Kepok Banana (*musa paradisiaca linn*) Peel Flour Addition as a Stabilizer on Chemical and Organoleptic Properties of Ice Cream

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## Abstact

The characteristic of pectin in kepok banana peel is also used as a stabilizer to make an ice cream.The research aim was todetermine the effect of kepok banana peel flour addition for producing ice cream which contains the best of chemical and organoleptic properties. Research method was arranged by using non factorial Random Complete Block Design (RCBD). It used single factor with six treatments and four replications. Treatments were 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, and 0.6% (w/v) of kepok banana peel flour.Furthermore, gelatin 0.5% was added as a reference of stabilizer which was used to determine organoleptic properties. Variable was observed and determined were organoleptic properties, overrun, emulsionstability and melting time. Subsequently, proximate analysis would be tested to the best treatment. Research results concluded that 0.1% (w/v) of kepok banana peel contains the best organoleptic properties: aroma score was 3.20 (bit of banana); flavor score was 3.85 (sweet); color score was 3.66 (brown); texture score was 4.00 (soft); and panelist acceptance was 3.48 (bit of like). Results of proximate analysis were: water content was 63.48%; protein content was 1.37%; fat content was 2.20%; ash content was 1.13%; fiber content was 1.56%; and carbohydrate content by different was 30.26%.

Keywords: kepok banana peel, flour, stabilizer, ice cream

## 1. Introduction

Banana is one of the horticultural commodities thathas a potential and high economic value. It has the highest plant area, productivity, and distribution in Indonesia. Total production of bananain 2013 was 5.359.126 tons and Lampung was 678.492 ton or contributed 12.66 % of national production (BPS, 2014). Kepok banana (*Musa Paradisiaca L.*) is a kind of banana which frequently used as a raw material for processing banana chips in Lampung. The production process of banana chips results peel as a waste yet it hashigh economic value. However, banana also produces waste which can be utilized.

Kepok banana peelcontains pectin compound.Ahda and Berry (2008) stated that pectin compound 10.10 % -11.93 % is containedin kepok banana peel. According to May (1990) in Hariyati (2006), pectin is used as a stabilizer in food processing. Thus, it can be used as astabilizer in the process of making ice cream. Kepok banana peel flour is can be added to the process of making ice cream as a stabilizer. There is no related previous research about using kepok banana peel as a stabilizer in the process of making ice cream. This research highlighted the effect of kepok banana peelflour addition for producing ice cream which contains best of chemical and organoleptic properties.

## 2. Materials and Methods

*2.1. Materials*

Kepok banana peel was obtained from Central Banana Chips Industry Bandar Lampung. Another materials were full cream milk powder (Indomilk), skim milk powder (Indomilk), sugar, water, and egg yolks. Chemicalanalysis was done by using hexan, concentrated $H_2SO_4$, $H_2SO_4$ 1.25 %, 1.25% NaOH;50% ;1 N, 0,02N HCl, $H_2BO_2$ , $Na_2S_2O_3$, citric acid, $CaCl_2$, distilled, indicators PP, and alcohol. This research also used knives, scales, stove, mixer, freezer, box freezer, pans, spoons, mixer stick, basins, thermometers, refrigerators, autoclaves, petri dishes, bottles, soxhlet, desiccator, furnace, porcelain dish, buchner funnel, measuring cups, oven, plate metal, kjeldahl flask, erlenmeyer, filter paper, pipette, analytical balance, glass tools and a set of organoleptic test.

*2.2. Methods*

Research method was arranged by using non factorial Random Complete Block Design (RCBD). It used single factor with six treatments and four replications. Treatments were 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, and 0.6% (w/v) of kepok banana peel flour.Furthermore, gelatin 0.5% was added as a reference of stabilizer which was used to determine organoleptic properties. Variable was observed and determined were organoleptic properties, overrun, emulsionstability and melting time. Proximate analysis would be tested to the best treatment.Data were analyzed by the analysis of variance to get the error variance.Subsequently, it was followed by using HSD parametric test at 5% level while the melting timewas analyzed descriptively.

*2.3. Research Procedure*

This research procedures were started by producing kepok banana peel flour (modified from Rois (2012)) as follows: a)one kg of kepok banana peel was washed with clean water and then drained; b) It was soakedin warm water for 10 minutes at a temperature of 70°C; c)dried using sun light; d). processed byusing disc mill and sieved using 40 mesh.

Next procedure, kepok banana peel flour as much as 300 grams were added for makingice cream (modified from Widyanti (2002)) as follows: a)raw materials such as water (70.9%), milk cream (10%), skim milk (7%), and sugar (12%) were mixed and homogenized formerly; b) it would be pasteurized at a temperature of 63°C for 30 minutes; c) Subsequently, it was added by 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, and 0.6% (w / v) kepok banana peel flour and gelatin 0.5% as a reference during pasteurization process; d) then added 2 egg yolk; e) materials were homogenized by using mixer for one hour; f) homogenized materials were given aging treatment in the freezer for hours; g) freezed in a deep freezer.

*2.4. Observation*

This research observed organoleptic properties (Watts et al., 1989), overrun(Marshall dan Arbuckle, 2000), emulsion stability (AOAC, 2005) and melting time(Roland et al., 1999).Proximate analysis that would be tested to the best treatmentwere water content (AOAC, 2005), fat content (Sudarmadji, 1984), protein content(Sudarmadji, 1984), ash content (AOAC, 2005), crude fiber content (Sudarmadji, 1984), and by different carbohydrate (Winarno, 1992).

## 3. Results and discussion

*3.1. Organoleptic Characteristics*

*1. Aroma*

The analysis of variance results is the addition of kepok banana peel flour has no effect to the aroma of ice cream, with average of 3.07.

Table 1. Score of ice cream aroma with the addition of kepok banana peel flour

| Treatments | Score | Note: |
|---|---|---|
| C3= The addition of banana peel flour 0.3% | 2.80 | 1 :Very typical of banana |
| C4= The addition of banana peel flour 0.4% | 3.05 | 2 : Typical of banana |
| C5= The addition of banana peel flour 0.5% | 3.03 | 3 : Bit typical of banana |
| C6= The addition of banana peel flour 0.5% | 3.08 | 4 : Not typical of banana<br>5 : Not very typical of banana |

Table 2. HSD parametric test at 5% levelon the flavor of ice cream with the addition of kepok banana peel flour

| Treatments | Score | Note: |
|---|---|---|
| C2= The addition of banana peel flour 0.2% | 3.09 [b] | 1 : Not very sweet |
| C3= The addition of banana peel flour 0.3% | 3.18 [b] | 2 : Not sweet |
| C4= The addition of banana peel flour 0.4% | 2.74 [b] | 3 : Bit of sweet |
| C5= The addition of banana peel flour 0.5% | 2.86 [b] | 4 : Sweet |
| C6= The addition of banana peel flour 0.6% | 2.71 [b] | 5 : Very sweet |
| HSD (0.05) = 0.49 | | |

*Note: Scores followed by the same letter is not differ significantly byHSDtest at 5% level*

Table 3. HSD parametric test at 5% levelon the color of ice cream with the addition of kepok banana peel flour

| Treatments | Score | Note: |
|---|---|---|
| C2= The addition of banana peel flour 0.2% | 3.85 [ab] | 1 : White Yellowish |
| C3= The addition of banana peel flour 0.3% | 3.99 [ab] | 2 : White Brownish |
| C4= The addition of banana peel flour 0.4% | 3.78 [ab] | 3 : Bit of brown |
| C5= The addition of banana peel flour 0.5% | 4.01 [ab] | 4 : Brown |
| C6= The addition of banana peel flour 0.6% | 4.33 [a] | 5 : Very brown |
| HSD (0.05) = 0.61 | | |

*Note: Scores followed by the same letter is not differ significantly byHSDtest at 5% level*

Based on the table, the addition of kepok banana peel flour does not affect the aroma of ice cream. Generally, ice ream has asugar and milk aroma (Rachmawati and Handajani, 2011). Banana peel flour can decrease milkaromaof ice cream. In addition, the starch hydrocolloids can slightly reduce the intensity of the aroma and flavor of a solution (Trenggono et al., 1989).

Aroma of banana peel due to the volatile compound that is contained in an evaporating banana peel. According to the research by Noorohmi (2010), the maceration of banana peel 13.305 % results an extract with dark brown color, less aroma of banana and gum scent. Types of volatile compounds contained in a yellow kepok banana peel based on Noorohmi (2010 ) ispentadekanoat acid and acid –9,12 oktadekadienoat.

*2. Flavor*

The analysis of variance resultsisthe addition of kepok banana peel flour affects the flavor of ice cream. Organoleptic results is between 2.71 - 3.85 (slightly sweet - sweet). Furthermore, HSD parametric test at 5% levelis presented in the following table. The table indicates that the treatment of C1 (0.1%) is different from treatment of C2 (0.2%), C3 (0.3%), C4 (0.4%), C5 (0.5%) and C6 (0.6%). Score of C1 is 3.85, which means flavor in ice cream is sweet. Each scores ofC2, C3, C4, C5, and C6 are respectively 3.09, 3.18, 2.74, 2.86, and 2.71. Flavor between the treatment of C2, C3, C4, C5, and C6 is no different with

scores ranging from 2.71 - 3:18. Treatment of C2, C3, C4, C5 and C4 have a bit of sweet flavor.

The differences of sweetness flavor in ice creamcaused by the amount of kepok banana peel flour. Kepok banana peel flour contains pectin compound that gives a bitter effect. Furthermore, banana peel flour decreases the sweetness flavor in ice cream. Trenggono et al. (1989) stated thatstarch hydrocolloids capableof reducing the intensity of the flavor of a solution. It is due to the ability of hydrocolloids that can coat on the tongue serves as a diffusion barrier.

*3. Color*

The analysis of variance resultsisthe addition of kepok banana peel flour affects the color of ice cream. Organoleptic result is between 3.66 - 4.33 (between bit of brown to brown). Furthermore, HSD parametric test at 5% levelis presented in the following table. Based on the table,treatment of C1 (0.1%) is different fromthe treatment of C6 (0.6%) yetto the treatment C2 (0.2%), C3 (0.3%), C4 (0.4%) and C5 (0.5% ) is not different. Scores of C1 is 3.66 (bit of brown) and a score of C6 is 4.33 (brown). Moreover, C6 treatment is not differentfrom thetreatment of C2, C3, C4, and C5, but in contrast with the treatment of C1.

The results show that the addition of kepok banana peel flour affects the color of ice cream. Color is one of the factors that influence consumer acceptance. According to Arbuckle (2000), color of ice cream should be interesting, homogen, and can represent the flavor.

The differences of color in ice cream caused by the amount of kepok banana peel flour.The increasing of kepok banana peel flour addition in ice cream causes more brown color. It is because kepok banana peel flour contains phenolic compounds that can makes a browning reaction (browning) due to oxygen exposure and heating process.Browning reaction is a reaction between oxygen and a phenol compound catalyzed by polyphenol oxidase. It results a formation of brown color (Winarno, 2001).

*4. Texture*

The analysis of variance results is the addition of kepok banana peel flour affects the texture of ice cream. Organoleptic result is between 2.83 – 4.00 (bit of hard until soft). Furthermore, HSD test at 5% levelis presented in the following table.

The table shows that treatment of C3 (0.3%) is different from the treatment of C1 (0.1%), C2 (0.2%), C5 (0.5%) and C6 (0.6%), yet it is same with treatment of C4 (0.4%). Score for the treatment ofC3 is 3.13 (bit of soft), while the score for another treatments are respectively C1 is 4 (soft), C2 is 3:38 (bit of soft), C5 is 2.93 (bit of soft), and C6 is 2.83 (bit of soft). Treatment of C5 and C6 is not different, but in contrast with treatments of C1, C2, C3.

Furthermore, treatment of C1 and C2are different toanother treatments. Results indicates that the addition of kepok banana peel flour affects the texture of ice cream. Kepok banana peel flour is non fat dried material that causesa difference of physicalcharacteristic, especially the texture of ice cream.

According to Elisabeth et al. (2007), the addition of skim milk and sweet potato steamed (non fat dried material) increases the viscosity of ICM (Ice Cream Mix). It narrows the mobility of water molecules in the ICM. Hence, the agitation process due to a limitation of incoming air in ICM causes the decreasing ofoverrun. Suprayitno et al., (2001) stated that low overrun causes ice cream texture same as a pudding, while high overrun causes ice cream texturebecome soft and melting rapidly.

Table 4. HSD parametric test at 5% level on the texture of ice cream with the addition of kepok banana peel flour

| Treatments | Score | Note: |
|---|---|---|
| C2= The addition of banana peel flour 0.2% | 3.38 [b] | 1 : Very hard |
| C3= The addition of banana peel flour 0.3% | 3.13 [c] | 2 : Hard |
| C4= The addition of banana peel flour 0.4% | 3.08 [cd] | 3 : Bit of soft |
| C5= The addition of banana peel flour 0.5% | 2.93 [d] | 4 : Soft |
| C6= The addition of banana peel flour 0.6% | 2.83 [d] | 5 : Very soft |
| HSD (0.05) = 0.22 | | |

*Note: Scores followed by the same letter is not differ significantly byHSDest at 5% level*

Table 5. HSD parametric test at 5% levelon the overall acceptance of ice cream with the addition of kepok banana peel flour

| Treatments | Score | Note: |
|---|---|---|
| C1= The addition of banana peel flour0.1% | 3.48 [a] | 1 : Very dislike |
| C3= The addition of banana peel flour 0.3% | 2.95 [b] | 2 : Dislike |
| C4= The addition of banana peel flour 0.4% | 2.80 [b] | 3 : Bit of like |
| C5= The addition of banana peel flour 0.5% | 2.65 [b] | 4 : Like |
| C6= The addition of banana peel flour 0.6% | 2.45 [b] | 5 : Very like |
| HSD (0.05) = 0.51 | | |

*Note: Scores followed by the same letter is not differ significantly by HSD test at 5% level*

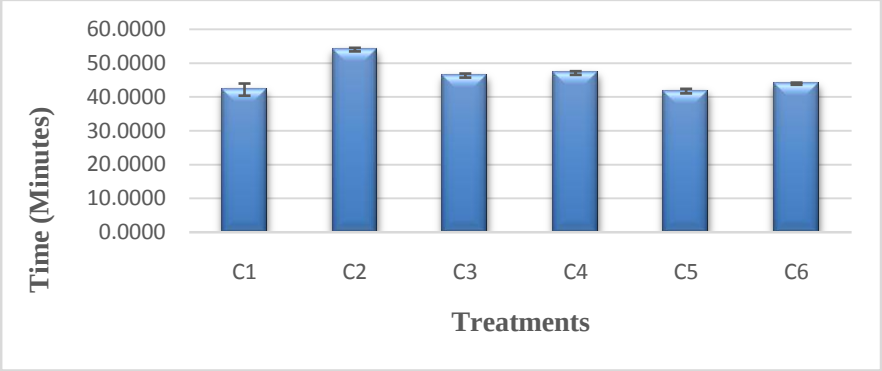


Fig 1. Melting Time of Ice Cream (Primary Data, 2016)

*5. Panelist Acceptance*

The analysis of variance resultsisthe addition of kepok banana peel flour affects the panelist acceptance of ice cream. Organoleptic result ofthe overall acceptance is between 2.45 - 3.48 (not like until bit of like). Furthermore,HSD test at 5% levelis presented in the following table.

The table shows that the treatment of C1 (0.1%) is different from the treatment of C2 (0.2%), C3 (0.3%), C4 (0.4%), C5 (0.5%) and C6 (0.6%). Scores on C1 treatment is 3.48, which means the overall acceptance of the ice cream is a bit like. Scores on the treatment of C2, C3, C4, C5, and C6 are respectively 2.74, 2.95, 2.80, 2.65, and 2.45. The overall acceptance of C2, C3, C4, C5, and C6 are no different with score between 2.45 - 2.95.

According to Francis (2003, in Neilsen, 2003) that color is an important factor that affects the overall acceptance of the product.Bad physical appearance of product can makes consumer reject without considering its nutrition value. However, the overall acceptance of panelist is affected byorganoleptic properties of ice cream.

Table 6. HSD parametric test at 5% level on the overrun of ice cream with the addition of kepok banana peel flour

| Treatments | Score |
|---|---|
| C2= The addition of banana peel flour 0.2% | 6.26 [a] |
| C3= The addition of banana peel flour 0.3% | 6.35 [a] |
| C4= The addition of banana peel flour 0.4% | 6.23 [a] |
| C5= The addition of banana peel flour 0.5% | 5.86 [b] |
| C6= The addition of banana peel flour 0.6% | 5.83 [b] |
| HSD (0.05) = 0.13 | |

*Note: Scores followed by the same letter is not differ significantly byHSDtest at 5% level*

Table 7. HSD parametric test at 5% levelon the emulsion stability of ice cream with the addition of kepok banana peel flour

| Treatments | Score |
|---|---|
| C1= The addition of banana peel flour0.1% | 60.64 [e] |
| C2= The addition of banana peel flour 0.2% | 62.52 [d] |
| C4= The addition of banana peel flour 0.4% | 65.50 [b] |
| C5= The addition of banana peel flour 0.5% | 68.56 [a] |
| C6= The addition of banana peel flour 0.6% | 68.95 [a] |
| HSD (0.05) = 0.77 | |

*Note: Scores followed by the same letter is not differ significantly byHSDtest at 5% level*

*6. Melting Time*

The results of melting time on ice cream with the addition of kepok banana peel flour arepresented in figure below.

Melting time is the time that is required by ice cream to melt formerlyat room temperature.According to Padaga and Savitri (2005), a shortmelting timein ice cream is less preferred because itmelts at room temperature in a short time. Yet, thelonger time is not also preferred because of the appearance of ice cream showssolid. The research results showed thatmelting time is over 40 minutes. According to Susilorini and Savitri (2006),melting time ofproper ice cream between 15-20 minutes. Longer melting time indicates the higher of solid material in the ice cream (Padaga and Savitri, 2005).

*7. Overrun*

Treatment ofC2 has a high melting time and decreases on C3, C4, C5, and C6 . This indicates that pectin compound in kepok banana peel flour works properly. Mechanism of pectin as astabilizer is done by spreadingfat globules thereby preventing a flock. Furthermore, it binds the water so that reducing the formation of crystals of ice cream during storage. Goff (2000) stated that stabilizer increases the viscosity and inhibitsmigration molecule of crystal nuclei, causesthe crystal size is limited and has a soft texture. Consequently, the increasing of stabilizer in ice cream results a soft texture and short melting time. However, lots of stabilizer is not desired. According Padaga and Savitri (2005), short melting timereflects that stabilizer is too much.

The analysis of variance resultsis the addition of kepok banana peel flour affectsoverrun of ice cream. The results of overrun is 5.76– 6.35%. Furthermore,HSD test at 5% levelis presented in the following table. The table shows thatthere is no difference between treatment of C1 (0.1%), C5 (0.5%) and C6 (0.6%), yet significantly differentfrom C2 (0.2%), C3 (0.3%) and C4 (0.4%). Treatment of C1, C5, and C6 are respectively 5.76%, 5.86% and 5.83%, while treatment of C2, C3, and C4 are respectively

6.26%, 6.35%, and 6.23%. The addition of kepok banana peel flour affects the overrun of ice cream. The difference of overrun scoresare caused by the amount of kepok banana peel flour on the ice cream.

Overrun shows that volume addition of ice cream is caused by air trapped in the ice cream mixture due to the agitation process. Overrun affects the texture and density that determines the quality of ice cream. Based on this research, overrun scoreis under the national standards established by the National of Standards Indonesia. Overrun score of reasearchis between 5.76 - 6.35% lower thannational standard (SNI NO. 01-3713-1995)isbetween 70-80% (industrial scale) and 30-50% (household).

Suprayitno et al. (2001) reported that low overrun in ice cream is caused by less of air trapped in the ice cream during the agitation. The addition of banana peel flour increaseseither solid materials or viscosity in ice cream. It is because of banana peel flour containsfibers due to its ability to bind water. According to Syafutri (2012) the increasing ofbanana peel flour causes the increasing of water bound yet decrease water content. It causes high viscosity and long melting time.It is because the starch has a functional properties as agelling agent (Trenggono et al., 1989).

*8. Emulsion Stability*

The analysis of variance resultsis the addition of kepok banana peel flour affectsthe emulsion stability of ice cream. The results of emulsion stability is between 60.64 - 68.95 %. Furthermore, HSD test at 5% levelis presented in the following table.

The table shows that treatment of C1 (0.1%) is different from all treatments. C2 treatment isalso different from all treatments. C3 treatment is also different from all treatments. C4 treatment is also different from all treatments. However there is no difference between C5 and C6, yet those treatments are different fromanother treatments. Score of emulsion stability C1, C2, C3, C4, C5, and C6 are respectively 60.64%, 62.52%, 63.73%, 65.50%, 68.56% and 68.95%.

The addition of kepok banana peel flour affects the emulsionstability of ice cream.The increasing ofkepok banana peel flour in ice cream also increases the emulsionstability of ice cream. The emulsionstability of ice cream indicates the resistence of ice cream to the separation of milkprotein and milk fat. Unstable emulsion causes the proteins would clump and settle in it, causing a separation between protein and fat (Arbuckle, 1986).

Arbuckle (1986) reported that the emulsion stability is affected by the type and amount of stabilizer, the size and homogenity of the fat globules, and the viscosity. The emulsion stability can be improved by making at globules become smaller and more homogen. Therefore, the addition of banana peel flourwhich contains pectin as a stabilizer is able to make high emulsion stability of ice cream.

Table 8. The summary of selection data based on the best treatment

| Parameter | Treatments | | | | | | Note: |
|---|---|---|---|---|---|---|---|
| | C1 | C2 | C3 | C4 | C5 | C6 | |
| Aroma | 3.20 | 3.28 | 2.80 | 3.05 | 3.03 | 3.08 | C1 :Banana Peel Flour 0.1% |
| Flavor | 3.85$^{a}$ | 3.09$^{b}$ | 3.18$^{b}$ | 2.74$^{b}$ | 2.86$^{b}$ | 2.71$^{b}$ | C2 :Banana Peel Flour 0.2% |
| Color | 3.66$^{b}$ | 3.85$^{ab}$ | 3.99$^{ab}$ | 3.78$^{ab}$ | 4.01$^{ab}$ | 4.33$^{a}$ | C3 :Banana Peel Flour 0.3% |
| Texture | 3.18$^{ab}$ | 2.66$^{b}$ | 3.58$^{a}$ | 3.33$^{ab}$ | 2.99$^{ab}$ | 2.94$^{b}$ | C4 :Banana Peel Flour 0.4% |
| Acceptance | 3.48$^{a}$ | 2.74$^{b}$ | 2.95$^{b}$ | 2.80$^{b}$ | 2.65$^{b}$ | 2.45$^{b}$ | C5 :Banana Peel Flour 0.5% |
| Melting Time | 42.13$^{c}$ | 53.95$^{a}$ | 46.28$^{b}$ | 47.03$^{b}$ | 42.18$^{c}$ | 43.88$^{c}$ | C6 :Banana Peel Flour 0.6% |
| *Overrun* | 5.76$^{b}$ | 6.26$^{a}$ | 6.35$^{a}$ | 6.23$^{a}$ | 5.86$^{b}$ | 5.83$^{b}$ | |
| Em.Stability | 60.64$^{e}$ | 62.52$^{d}$ | 63.73$^{c}$ | 65.50$^{b}$ | 68.56$^{a}$ | 68.95$^{a}$ | |

Table 9. Proximate analysis of ice cream with the addition of kepok banana peel flour 0.1%

| Parameter | Value (%) |
|---|---|
| Water content | 63.48 |
| Protein content | 1.37 |
| Fat content | 2.20 |
| Ash content | 1.13 |
| Fiber content | 1.56 |
| Carbohydrate by different | 30.26 |

Table 10. The quality of ice cream nutrition

| No. | Test Criteria | Unit | Terms |
|---|---|---|---|
| 1. | Fat | % w/w | Minimum 5.0 |
| 2. | Sugar calculated as sucrose | % w/w | Minimum 8.0 |
| 3. | Protein | % w/w | Minimum 2.7 |

Source: BSN-SNI 01-3713-1995

*9. Selection of Best Treatment*

This research determined the best treatment based on the results of the organoleptic (aroma, flavor, color, texture and overall acceptance), melting time, overrun, and emulsionstability. The summary of selection data based on the best treatment as follows. The best treatment was determinedby indentifying two aspects: organoleptic (aroma, flavor, color, texture, and overall acceptance) and laboratory test (melting time, overrun, and emulsion stability). It usedstar notation method is by appraising the number of stars. Stars were given to the best letters which is categorized based on the parameters as well as the closest to exist standard. It was also given to the letter which is not differ from the best parameter. The researchreportedthat C1 treatment is the greatest. However, the laboratory test of this product has to be developed before commercializingin order to get as well as the Indonesian National Standard of ice cream products.

*10. Proximate Analysis*

Proximate analysis was identified to determine the nutrition of ice cream which is added by kepok banana peel flour as much as 0.1% (25 grams). Proximate analysis were water content, protein content, fat content, ash content, fiber content and carbohydrate by different. It was summarized in Table 9.

Table shows that the treatment of C1 contains water content of 63.48%, protein content of 1.37%, fat content of 2.20%, ash content of 1.13%, fiber content of 1.56%, and carbohydrateby different of 30.26%.

Comparing to the table 10, the ice cream nutrition with the addition of kepokbanana peel flour as much as 0.1 % has a protein and at lower than standard. It was caused by the slightly protein and fat content due to less of kepok banana peel flour. Furthermore, the fiber content in kepokbanana peel flour can bind fat content (Tensiska, 2008) so that can be counted in fat analysis.

## Conclusion

This research concluded that 0.1% (w/v) of kepok banana peel contains the best organoleptic properties: aroma score was 3.20 (bit of banana); flavor score was 3.85 (sweet); color score was 3.66 (brown); texture score was 4.00 (soft); and panelist acceptance was 3.48 (bit of like). Results of proximate analysis were: water content was 63.48%; protein content was 1.37%; fat content was 2.20%; ash content was 1.13%; fiber content was 1.56%; and carbohydrate content by different was 30.26%.

## References

Ahda, Y. and Berry, S.H. 2008. Pengolahan Limbah Kulit Pisang Menjadi Pektin Dengan Metode Ekstraksi. *J.Teknik Kimia*. Universitas Diponegoro.

AOAC. 2005. *Official Method of Analisis of the Associates of Official Analytical Chemist*. AOAC. Inc, New York 1141 pp.

Arbuckle, W.S. 1986. *Ice Cream.*Avi Publishing Company. Inc West Port, Connecticut.

Arbuckle, W.S. 2000. *Ice Cream Third Edition.* Avi Publishing Company. Inc West Port, Connecticut.

BadanPusatStatistik. 2014. *ProduktivitasPisangNasional*. Badan Pusat Statistik, Jakarta.

BadanStandarisasiNasional. 1995. *Persyaratan Mutu Es Krim*. Badan Standarisasi Nasional, Jakarta.

Bodyfelt, F.W., Tobias J, Trout G.M. 1988. *The Sensory Evaluation of Dairy Products*. AVI Publishing, New York.

Desroiser, N.W. 1988. *TeknologiPengawetanPangan*. UI-Press, Jakarta

Elisabeth, D.A., M. A. Widiyaningsih and I. K. Kariada. 2007. *Pemanfaatan Umbi Jalar sebagai Bahan Baku EsKrim*. Balai Pengkajian Teknologi Pertanian (BPTP) Bali, Bali.

Francis, F. J. 2003. *Color Analysis.* in: Neilsen, S. S. 2003. *Food Analysis 3rd Edition.* Kluwer Academic, New York.

Goff, H.D. 2000. Controlling ice cream structureby examining fat protein interactions**.** *J. Dairy Technology, Australia*.

Hariyati, M.N. 2006. Ekstraksi dan Karakterisasi Pektin dari Limbah Proses Pengolahan Jeruk Pontianak (*Citrus nobilisvarmicrocarpa*). (Skripsi). Institut Pertanian Bogor. Bogor.

Harris, A. 2011. Pengaruh Substitusi Ubi Jalar (*Ipomeabatatas*) dengan Susu Skim Terhadap Pembuatan Es Krim. (Skripsi). UniversitasHasanuddin, Makassar.

Hartoyo,. 2003. *The dan Khasiatnya Bagi Kesehatan:Sebuah Tinjauan Ilmiah.* Kanisius. Yogyakarta.

Marshall, R.T. and W.S. Arbuckle. 1996**.** *Ice Cream, 5th Edition.* International Thompson Publishing, New York.

Noorohmi, U. 2010. Penentuan Senyawa Volatil Dari Kulit dan Daging Buah Pisang Kepok Kuning (*Musabalbisiana)* Secara Kromatografi Gas - Spektrometer Massa (GC-MS). (Skripsi). Universitas Negeri Yogyakarta, Yogyakarta.

Padaga, M and M.E. Sawitri. 2005. *EsKrim yang Sehat*. TrubusAgrisarana, Surabaya.

Rachmawanti, A.D and S. Handajani. 2011. EsKrimUbiUngu (*Ipomoea batatas*) :TinjauanSifatSensoris, Fisik, Kimia, danAktivitasAntioksidannya. (Skripsi). InstitutPertanian Bogor. Bogor

Rois, F. 2012. Pembuatan Mie TepungKulitPisangKepok (KajianSubstitusiTepungKulitPisangKepokpadaTepungTerigudanPenambahanTelur. (Skripsi). Universitas Pembangunan Nasional "Veteran". Surabaya.

Roland, A.M., L.G. Phillips dan K.J. Boor. 1999. Effect of fat content on the sensory properties, melting, colour and hardness of ice cream. *J. Dairy Sci*. 82: 32-38.

Sudarmadji, S. et al. 1984. Analisa Bahan Makanan dan Pertanian. Liberty, Yogyakarta.

Suprayitno, E., H. Kartikaningsih, and Rahayu. 2001. Pembuatan Es Krim Menggunakan Stabilisator Natrium Alginate darisargassum sp. *J.MakananTradisional Indonesia ISSN: 1410-8968*. 1(3): 23-27.

Suparmi andHarka, P. 2012. Aktivitas Antioksidan Ekstrak Kasar Pigmen Karotenoid Pada Kulit Pisang Ambon Kuning (*musa parasidiacasapientum l.*):potensi Sebagai Suplemen Vitamin a. *J.Ilmiah Formasi*. 4 (1): 84-86.

Susilorini, T.E and M.E. Sawitri. 2006. ProdukOlahanSusu. PenebarSwadaya, Jakarta.

Syafutri, M.I. 2012. Karakteristik Es Krim Hasil Modifikasi Dengan Formulasi Bubur Timun Suri dan Sari Kedelai. *J.Teknol. danIndustriPangan*. 23(1): 17–22.

Tensiska. 2008. *SeratMakanan*. UNPAD, Bandung.

Trenggono, Sutardi, Haryadi, Suparmo, A. Murdiati., S. Sudarmadji, K. Rahayu, S. Naruki and M. Astuti. 1989. *BahanTambahanPangan*. Universitas Gajah Mada, Yogyakarta.

Watts, B. M., G. L. Ylimaki, L. E. Jeffery and L. G. Elias. 1989. *Basic Sensory Methods for Food Evaluation*. International Development Research Center, Canada.

Widyanti, S.M. 2002. Pengaruh Penggunaan Jenis Minyak Sawit dan Bahan Penstabil Terhadap Karakteristik Es Krim. (Skripsi). Universitas Lampung, Bandar Lampung.

Winarno, F.G. 1992. Kimia Pangan. Gramedia, Jakarta.

Winarno, F.G. 2001. Kimia Pangan. PT GramediaPustakaUtama, Jakarta.

Yosephine A., V. Gala, A.Ayucitra, and E.S Retnoningtyas. 2012. Pemanfaatan Ampas Tebu dan Kulit Pisang Dalam Pembuatan Kerta Serat Campuran. *J.Teknik Kimia Indonesia*. 11(2): 94-100

# The effect of soil submersion duration and ameliorant types on growth and yield of shallot at Brebes Regency

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## Abstract

Brebes Regency is the central of shallot production in Central Java. However, shallot productivity in this Regency has been decreasing from 16 t/ha to 12.23 t/ha. This resulted from soil depletion since the cultivation systems were not maximal, continuous used of unbalance inorganic fertilizers and over doses of synthetic pesticide applications. Submersion of land and application of soil ameliorants might solve the problem. This research aimed to know the effect of soil submersion duration, the soil ameliorant types which was effective, and their interaction on growth and production of shallot. The research was conducted in Wanasari village, Wanasari district, Brebes. It was conducted from Mei until August 2016. This was a 2 x 2 factorial experiment with 4 replications arranged in a randomized complete block designs. The first factor was duration of land submersion i. e. 12 hours or 24 hours. The second factor was soil ameliorant types i. e. chicken litter or zeolit. Observed variabels were plant height, leave numbers, bulb numbers per plot, bulb numbers per cluster, diameter of the biggest bulb in a cluster, fresh weight of bulb per cluster, fresh weight of plant per cluster, fresh weight of bulb per plot, fresh weight of plant per plot, weight bulb per cluster after sun drying for 3 days,weight plant per cluster after sun drying for 3 days, weight of bulb per plot after sun drying for 3 days, weight of plant per plot after sun drying for 3 days, weight of plant per cluster after sun drying for 7 days, weight of bulb per cluster after sun drying for 7 days. Result found that the duration of submersion, the ameliorant types, and their interaction did not significantly affect the growth and yield variabels of shallot.

Keywords: duration of submersion, ameliorant, shallot

## 1. Introduction

The potential development of the cultivation of shallot has good prospects since shallot is used in household as vegetable spices with no substitutes which is continually used as seasonings for daily dishes, traditional medicine and health benefits, such as anti-cancer agent, substitution for antibiotics, blood pressure lowering, cholesterol reduction, and blood sugar decrease, in addition, shallot also has high economic value [1, 2].

The Regency of Brebes is one of regencies in Central Java where shallot is cultivated and produced, which is capable to produce and supply the needs of shallot in Indonesia up to 50 to 51.4% [3, 4]).

The shallot production in The Regency of Brebes in 2014 reached 375.97 thousand tons with a total harvest area of 30.95 thousand hectares and the productivity in 2013 reached 12.23 tons per hectare [5], while the potential of shallot productivity could reach 20 tons per hectare [3]. Meanwhile, the productivity of shallot in 1970s could reach 16 tons per hectare [6].

The decreased productivity of shallot is caused by the cultivation system which is not

optimal and continual excessive use of inorganic fertilizers [7]. Nowadays, farmers intensively cultivate shallot for commercial purpose. Farmers believe that the use of synthetic chemical fertilizers and pesticides in the cultivation of plants is a means to improve production and to avoid crop failure. However, farmers rely heavily on the use of inorganic fertilizers that applied continuously and exessively in a long period of time with high dosage that cause an increase of heavy metal content, environmental pollution, deterioration and decline in soil fertility as a result of the imbalance of nutrients or the shortage of other nutrients and the more low soil organic matter content [8, 9, 10, 11]. In addition, farmers always apply synthetic pesticides unwisely. It means that the use of synthetic pesticides is increasing which causes pesticides residues in agricultural products gives negative impacts on the environment and human health [12]. Previous study [13] reported the frequency of synthetic pesticides usage by the farmers in Brebes regency in shallot cultivation is too high namely once every 2-3 days by mixing and intermixing various types of pesticides at the same time and the pesticide residues are still exist either on the ground or on the shallots and the pesticides with the active ingredient of chlorpyrifos contains the value of residues above the maximum limit of pesticide residues in shallot bulbs.

The anticipation of this conditions and the alternative step in restoring soil fertility and environmental damage can be done by the process of submersion and the application of soil conditioner materials. The process of submerging the soil may lead to the dissolution of substances that are soluble and leached by submersion [14].

Other technologies to improve soil fertility and environmental damage are the application soil conditioner materials. The soil conditioner materials are natural ingredients that can improve the properties of soil physically, chemically, and biologically, so that it can support the growth of plants [15, 16]. The soil conditioner material can be derived from plants, manure, zeolites, and clays [15, 17].

The purpose of this study was to determine the effect of soil submersion duration, types of soil ameliorants and their interaction to the growth and production of shallot in The District of Brebes.

## 2. Material and Methods

The research was conducted in the village of Wanasari, District Wanasari, Brebes from May to August 2016. This was a 2 x 2 factorial experiment with 4 replications arranged in a randomized complete block designs. The first factor was duration of land submersion i. e. 12 hours or 24 hours. The second factor was soil ameliorant types i. e. chicken litter or zeolit. Observed variabels were plant height, leave numbers, bulb numbers per plot, bulb numbers per cluster, diameter of the biggest bulb in a cluster, fresh weight of bulb per cluster, fresh weight of plant per cluster, fresh weight of bulb per plot, fresh weight of plant per plot, weight bulb per cluster after sun drying for 3 days,weight plant per cluster after sun drying for 3 days, weight of bulb per plot after sun drying for 3 days, weight of plant per plot after sun drying for 3 days, weight of plant per cluster after sun drying for 7 days, weight of bulb per cluster after sun drying for 7 days. Result found that the duration of submersion, the ameliorant types, and their interaction did not significantly affect the growth and yield variabels of shallot.

## 3. Result and Discussion

Statistical analysis it showed that submersion of soil for 12 hours or 24 hours before cultivating and planting of shallots has similar effect on all observed variables (Table 1).

Table 1. Effect of soil submersion duration on growth and yield of shallots

| Treatments | Variable of Observation | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TT (cm) | JD (unit) | JA (unit) | JUPR (unit) | DUPR (cm) | BBUR (g) | BBTR (g) | BBUP (kg) | BBTP (g) | BKUR (g) | BKTR (g) | BKUP (g) | BKTP (g) | BKTRP (g) | BKURP (g) |
| P1 | 38,47 | 26,7 | 5,5 | 6,3 | 2,75 | 70,25 | 115 | 3,68 | 7343,75 | 46 | 59,63 | 2462,5 | 3087,5 | 45,65 | 39,52 |
| P2 | 38,44 | 27,8 | 5,75 | 6,65 | 2,86 | 67,25 | 126,25 | 3,24 | 7037,5 | 48,25 | 62,63 | 2456,25 | 2863,75 | 47,59 | 39,40 |
| F count | 0,00094$^{tn}$ | 0,25$^{tn}$ | 0,15$^{tn}$ | 0,24$^{tn}$ | 0,98$^{tn}$ | 0,32$^{tn}$ | 1,75$^{tn}$ | 1,19$^{tn}$ | 0,19$^{tn}$ | 0,15$^{tn}$ | 0,15$^{tn}$ | 0,00045$^{tn}$ | 0,46$^{tn}$ | 0,21$^{tn}$ | 0,00067$^{tn}$ |
| F table 5% | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 |
| F table 1 % | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 |

Remarks: tn = *not significantly different*, P1 = soil *submersion for 12 hours*, P2 = *soil submersion for 24 hours*, TT = *height of plant*, JD = *number of leaves*, JA = *number of tillers*, JUPR = *number of bulbs per cluster*, DUPR = *diameter of bulbs per cluster*, BBUR = *fresh weight of bulbs per cluster*, BBTR = *fresh weight of plant per cluster*, BBUP = *fresh weight of bulbs per plot*, BBTP = *fresh weight of plant per plot*, BKUR = *weight of bulbs per cluster after 3 days of sun drying*, BKTR = *weight of plants per cluster after 3 days of sun drying*, BKUP = *weight of bulbs per plot after 3 days of sun drying*, BKTP = *weight of plants per plot after 3 days of sun drying*, BKTRP = *weight of plants per cluster after 7 days of sun drying*, BKURP = *weight of bulbs per cluster after 7 days of sun drying*

Table 2. Effect of soil ameliorants on growth and yield of shallots

| Treatments | Variable of Observation | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TT (cm) | JD (unit) | JA (unit) | JUPR (unit) | DUPR (cm) | BBUR (g) | BBTR (g) | BBUP (kg) | BBTP (g) | BKUR (g) | BKT R (g) | BKUP (g) | BKTP (g) | BKTRP (g) | BKURP (g) |
| T1 | 39,08 | 27,05 | 5,6 | 6,4 | 2,77 | 66,75 | 122,25 | 3,57 | 7330 | 45,13 | 57,13 | 2481,25 | 3150 | 44,06 | 36,58 |
| T2 | 37,84 | 27,43 | 5,65 | 6,55 | 2,84 | 70,75 | 119 | 3,35 | 7051,25 | 49,13 | 65,13 | 2437,5 | 2801,25 | 49,18 | 42,33 |
| F count | 1,18$^{tn}$ | 0,031$^{tn}$ | 0,006$^{tn}$ | 0,045$^{tn}$ | 0,42$^{tn}$ | 0,57$^{tn}$ | 0,15$^{tn}$ | 0,30$^{tn}$ | 0,16$^{tn}$ | 0,46$^{tn}$ | 1,05$^{tn}$ | 0,022$^{tn}$ | 1,12$^{tn}$ | 1,44$^{tn}$ | 1,53$^{tn}$ |
| F table 5% | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 |
| F table 1 % | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 |

Remarks: tn = *not significantly different*, T1 = *chicken litter*, T2 = *zeolite*, TT = *height of plant*,JD = *number of leaves*, JA = *number of tillers*, JUPR = *number of bulbs per cluster*, DUPR = *diameter of the largest tuber per cluster*, BBUR = *fresh weight of bulbs per cluster*, BBTR = *fresh weight of plants per cluster*, BBUP = *fresh weight of bulbs per plot*, BBTP = *fresh weight of plants per plot*, BKUR = *weight of bulbs per cluster after 3 days of sun drying*, BKTR = *weight of plants per cluster after 3 days of sun drying*, BKUP = *weight of bulbs per plot after 3 days of sun drying*, BKTP = *weight of plants per plot after 3 days of sun drying*, BKTRP = *weight of plants per cluster after 7 days of sun drying*, BKURP = *weight of bulbs per cluster after 7 days of sun drying*

Table 3. Effect of soil submersion durations and soil ameliorant types on growth and yield of shallots

| Treatments | Variable of Observation | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TT (cm) | JD (unit) | JA (unit) | JUPR (unit) | DUPR (cm) | BBUR (g) | BBTR (g) | BBUP (kg) | BBTP (g) | BKUR (g) | BKTR (g) | BKUP (g) | BKTP (g) | BKTRP (g) | BKURP (g) |
| P1T1 | 38,75 | 26,95 | 5,55 | 6,3 | 2,68 | 66 | 110 | 3,93 | 7662,5 | 45 | 56,25 | 2587,5 | 3325 | 41,5 | 34,44 |
| P1T2 | 38,2 | 26,45 | 5,45 | 6,3 | 2,83 | 74,5 | 120 | 3,44 | 7025 | 47 | 63 | 2337,5 | 2850 | 49,79 | 44,59 |
| P2T1 | 39,4 | 27,15 | 5,65 | 6,5 | 2,87 | 67,5 | 134,5 | 3,21 | 6997,5 | 45,25 | 58 | 2375 | 2975 | 46,62 | 38,73 |
| P2T2 | 37,48 | 28,4 | 5,85 | 6,8 | 2,85 | 67 | 118 | 3,26 | 7077,5 | 51,25 | 67,25 | 2537,5 | 2752,5 | 48,56 | 40,07 |
| F count | 0,36$^{tn}$ | 0,17$^{tn}$ | 0,06$^{tn}$ | 0,04$^{tn}$ | 0,75$^{tn}$ | 0,72$^{tn}$ | 2,43$^{tn}$ | 0,44$^{tn}$ | 0,27$^{tn}$ | 0,12$^{tn}$ | 0,03$^{tn}$ | 0,49$^{tn}$ | 0,15$^{tn}$ | 0,55$^{tn}$ | 0,89$^{tn}$ |
| F table 5% | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 | 5,12 |
| F table 1 % | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 | 10,56 |

Remarks: tn = *not significantly different*, P1 = *soil submersion for 12 hours*, P2 = *soil submersion for 24 hours*, T1 = *chicken litter*, T2 = *zeolite*, TT = *height of plant*, JD = *number of leaves*, JA = *number of tiller*, JUPR = *number of tuber per cluster*, DUPR = *diameter of the biggest bulbs per cluster*, BBUR = *fresh weight of bulbs per cluster*, BBTR = *fresh weight of plants per cluster*, BBUP = *fresh weight of bulbs per plot*, BBTP = *fresh weight of plants per plot*, BKUR = *weight of bulbs per cluster after 3 days of sun drying*, BKTR = *weight of plants per cluster after 3 days of sun drying*, BKUP = *weight of bulbs per plot after 3 days of sun drying*, BKTP = *weight of plants per plot after 3 days of sun drying*, BKTRP = *weight of plants per cluster after 7 days of sun drying*, BKURP = *weight of bulbs per cluster after 7 days of sun drying*

This indicated that Soil submersion for 12 hours or 24 hours before cultivating the soil and planting the shallot could dissolve and wash substances that have a negative impact on the growth of plants.

According previous report [14], the submersion treatment will lead to the dissolution of soluble substances and undergo washing by submersion.

In addition, regular watering was done during cultivation every morning and afternoon, so it provided the same effect on improvement of soil conditions and the provision of water needed by plants for growth and production of shallot bulbs. Others [9] reported that the availability of water, $CO_2$ and sunlight will support the process of photosynthesis.

Statistical analysis showed that the application of chicken litter or zeolite as soil ameliorant has similar effect on all observed variables (Table 2.). Providing organic materials when cultivating the soil is a must because it becomes one of the factors that play a role in the success of plant cultivation [18].

The chicken litter is solid organic fertilizer which contains complete and sufficient nutrients such as macro and micro nutrients, improves the soil structure, increases the content of nutrients, develops the soil organic matter, raises the living conditions in the soil, increases the capacity of water holding and the capacity of cation exchange which lead to the better growth of the root, so that they support the growth of plants [19, 20]. Organic fertilizer is natural, does not damage the soil, contains all the nutrients, both macro and micro, needed by plants, improves endurance and absorbs water, develops the activity of soil microbiology, enhances the cation exchange capacity, restores the soil structure, gives the better soil aeration, and prevents compaction [9, 21].

Zeolite constitutes an aluminosilicate mineral which forms the micro pores that can hold some kinds of molecules including cations and water. Zeolite has the ability as absorbent, catalyst and booster of the cation exchange capacity [22]. Zeolite is one of the soil conditioner material that has large absorption, capability to store water in the soil, and ability to improve soil fertility[23].

Statistical analysis showed that the interaction between the soil submersion duration and the application of the soil ameliorant types had no significant effect on all observed variables (Table 3.). This might resulted from that both chicken litter and zeolite as soil ameliorant were improved the fertility of the soil, absorbed water, increased the cation exchange capacity, and hindered the process of evaporation from the soil, so plants do not experience a lot of water loss [8, 15, 17, 24].

## Conclusion

It can be concluded that submersion of soil for 12 hours or 24 hours before cultivating and planting had similar effect on growth and yield of shallots; chicken litter or zeolite as soil ameliorant had similar effect on growth and yield of shallots. There was no interaction effect of soil submersion duration and soil ameliorant types on growth and yield of shallots

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## References

1. Berson, Mariati, Rosita. 2015. Production of Samosir Shallot Simanindo Accession against The Concentrations of GA3 and The Prolonged submersion in the Highlands of Samosir. Online Journal Agroecotechnology, 3, …. - ….
2. Novayana, D., Sipayung, R., and Barus, A. 2015. Response of Growth and Production of Shallot (Allium ascalonicum L.) against Type of Mulch

and Chicken Manure. Online Journal Agroecotechnology, 3, …. - ….

3. National Development Planning Agency. 2013. Preliminary Study: Nasional Medium Term Development Plan (RPJM) Field of Food and Agriculture 2015-2019. Directorate of Food and Agriculture, Ministry of National Development Planning / National Development Planning Agency.
4. Rachmat, M., Sayaka, B., and Muslim, C. 2015. Production, Trade and Prices of shallot……. ……..
5. BPS of Central Java Province. 2015. Production of Hot Pepper, Cayenne pepper, and Red Shallot in 2014 Central Java Province. Official Statistics. 56/08/33 Th. IX. …….. ……..
6. Haq, M. M. N., and Umarie, I. 2015. Response of Some Varieties of Shallot and Duration of GA3 Submersion on the Growth and Yield. Agritrop Journal of Sciences of Agriculture. Faculty of Agriculture,…. , …. - ….
7. Samad, S. 2012. Effects of Organic Fertilizer on the Growth and Production of Shallots in Dryland of Lowlands. Faculty of Agriculture, University of Khairun. ……..
8. Pangaribuan, S. H., Yasir, M., and Utami, N. K. 2012. Impact of Livestock Manure of Bokashi in Reduction of Inorganic Fertilizer Application on Tomato Cultivation. J. Agron Indonesia, 40, …. - ….
9. Anisyah, F., R. Sipayung, C. Hanum. 2014. Growth and Production of Shallot by Giving Organic Fertilizer. Online Journal Agroekoteknologi, 2, …. - ….
10. Nurjaya, Jihan, E., and Saeni, M. S. 2006. Effect of Ameliorant against *Pb* Degree of Soil, Its Absorbance and Shallot Crop on Inceptisol. Journal of Agricultural Sciences Indonesia, 8, …. - ….
11. Simmamora, A.L.B., T. Simanungkalit, J. Ginting. 2013. Response of Growth and Production of Shallot (*Allium ascalonicum* L) on the Provision of Vermicompost and Rabbit Urine. Online Journal Agroecotechnology, 2, …. - ….
12. Hartati, C.S., A. Permanasari, Zackiyah. 2011. Simultaneous Adsorption of Chitosan-Bentonite against Metal Ion and Pesticide Residue in Drinking Water by Engineering Batching Technique. National Seminar Papers of Chemical Unjani. Pp. …. - ….
13. Badruddin, U., and Suryotomo, B. 2015. Study of Pesticide Residues on Cultivation of Shallot (*Allium ascalonicum L*) by The Application of Soil Conditioner Materials and The Botanical Pesticide to Achieve Environmentally Friendly Agriculture. Research Report. Faculty of Agriculture, University of Pekalongan
14. Suarti, B., Fuadi, M., and Siregar, B.H. 2013. Making Starch from Durian Seeds Through The Addition of Sodium Metabisulphite and Prolonged Immersion. Agrium., 18, …. - ….
15. Mustafa, W. S., Izzati, M., and Saptiningsih, E. 2012. Interaction between Soil Conditioner of *Hydrilla verticillata* Royle and *Salvinia molesta* Mitchell on Field Capacity of Sandy Soil and Clay as well as The Growth of Green Bean (Vigna radiata L.). Bulletin of Anatomy and Physiology, XX, …. - ….
16. Nurhayati. 2011. Effect of Ameliorant to Some Chemical Properties of Peat Soil and Growth Vegetative Plant of Soybean (*Glycine max* L. Merrill). Agrium.,16, …. - ….
17. Setyono, B and Suradal. 2006. Feasibility of Shallot Farming in Coastal Sandy Soil by Amelioration Technology in Bantul Regency of he Special Regions of Yogyakarta. Assessment Institute of Agricultural Technology, Yogyakarta.
18. Pratiwi, Santoso, E., and Turjaman, M. 2012. Determination of Dose of Conditioner Materials (*Ameliorant*) for Soil Improvement of Quartz Sand Tailings as Growing Media of Forest Plants. *Journal of* Forest Research and Nature Conservation., 9, …. - ….
19. Rahman, A., R. Sipayung, T. Simanungkalit. 2013. Growth and Production of Shallot (*Allium ascalonicum* L.) with the Application of Chicken Manure and EM4 (Effective Microorganisms4). Online Journal Agroecotechnology, 1, …. - ….
20. Rauf, F., Bahua, M. I., and Guarantee, F. S. 2014. Effectiveness Test of Potassium Fertilizer and Organic Fertilizer on Growth and Production of Peanut *(Arachis hypogeae* L.). Department of Agrotechnology, Faculty of Agricultural Sciences, State University of Gorontalo
21. Yuliana, Rahmadani, E., and Permanasari, I. 2015. Application of Beef and Chicken Manure on the Growth and Yield of Ginger Crops (*Zingiber officinale* Rosc.) In Peat Media. Journal of Agrotechnology, 5, …. - ….
22. Goenadi, D. H. 2004. Zeolite Processing Technology into Materials with High Economic Value. Journal Zeolite Indonesia, 3, …. - ….
23. Sofan, F. M. I. S., Budiyanto, G., and Main, N. A. 2016. Application of Zeolite + Night Soil To Improve The Growth Quality and The Yield of Shallot (*Allium ascalonicum*) in The Coastal Sandy Soil. Agrotechnology Study Program, Faculty of Agriculture, University of Muhammadiyah Yogyakarta.

# Disease progress of Stewart's Wilt (*Pantoea stewartii* subsp. *stewartii*) on sweet corn

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## Abstract

*Pantoea stewartii* subsp. *stewartii* cause Stewart's wilt disease in maize. This pathogen is still relatively new reported in Indonesia. Based on the Regulation of the Minister of Agriculture of the Republic of Indonesia Number 51 / Permentan / Kr.010 / 9/2015 On Plant Quarantine type, has been determined that this organism as the organism pest plant quarantine A2 category. Disease progression of the disease is the basis / principle for designing control strategies of the disease and reduces the risk of the disease. The research objective is to obtain a model of disease progress and spread pattern of Stewart's wilt on sweet corn in the field. Research in the form of field trials using a plot measuring 5.00 x 11.00 m with 6 replications and have not used Experimental Design. Observation were disease incidence and disease severity that measure in every 7 days. Model of disease progress was testing by three theoretical models: Logistic, Monomolekuler, and Exponential that analyzed by using $R^2$ and MSR. The spreat pattern of the disease was using Run analyzed and testing by using Z test. Result of the research has found that model of disease progress is Monomolekuler and spreat pattern of disease is Random.

Keywords: disease progress, model, spreat pattern, *P. stewartii* subsp. *Stewartii*

## 1. Introduction

Stewart's wilt disease (*Pantoea stewartii* subsp. *stewartii*) on maize was the new reported to exist in Indonesia. Furthermore, in West Sumatra have identified three isolates from several centers of maize that are *P. stewartii* subsp. *stewartii* strain R1-104 isolates LA, *P. stewartii* subsp. *stewartii* strain R1-132 isolates PR1, and *P. stewartii* subsp. *stewartii* strain ATCC 29923 isolates PR2 ( Desi et al., 2014).

These pathogens classified as important because it is seedborne disease (Baylor Collage of Medicine, 2006; EPPO Buletin, 2006; Munkvold, 2001; Pataky and Ikin, 2003; Plant and Pest Diagnostic Laboratory, 2012). The yield loss ranged from 40-100% in sweet corn susceptible and infected in phase V5 (Pataky and Michener, 2004) and can also be spread by insect vectors especially flea beetle (*Chaetocnema pulicaria*) (Pataky et al., 2002; Esker and Nutter, 2002). Flea beetles can survive the winter in other plants such as: orchas grass, crab grass, witch grass, sudan grass, foxtail, barley, wheat, and oats ( Gray, 2008).

Control against to Stewart's wilt disease was reported only abroad, among them: practical management to reduce the risk of diseases such as: minimizing the initial infection, the use of insecticides seed treatment, look for resistance genes (Liu, 2010) and predicts the risk of failure disease in the field using a system of forecasting temperatures in December, January, and February (Gray, 2008; Munkvold and Rice,

1997; Pollock, 2002). Mid-term prediction conducted in Taiwan against to *P. stewartii* subsp. *stewartii* is 2 months before planting (Xu et al., 2009).

Management of disease risks associated with the disease progress is usually determined by the increased incidence and severity of disease based on the time and place (temporal-spatial), is fundamental importance in epidemiology (Subekti et al., 2003). Understanding of disease progress is the basis/foundation for the development of methods in an attempt to control diseases, such as the development of forecasting models, determining the optimal timing of pesticide use and the timing of planting to reduce the risk of disease (Rivai, 2009). Prediction of yield loss as a result of measurement of intensity of the disease is usually approached from relationship between them. Data observation of disease severity and yield loss caused, very difficult to understand unless treated within the framework of the quantitative relationship according to the relevant mathematical models. One form of the relationship is a critical time. Critical time is not always a point of time (days) in the growth phase of the plant, but it can be a short-term (more than one day) in the growth phase of the plant (Rivai, 2009).

Until now, limited research related to strategies of control Stewart's wilt disease that may be answer from disease progression, like development by time and spreat by space, This study aims to obtain a model of the disease progress and spreat pattern of disease in the field.

## 2. Material and Methods

Research was conducted in Nagari Koto Baru, Kecamatan Luhak Nan Duo, Kabupaten Pasaman Barat from March until Juni in 2015. Research in the form of field trials using a plot measuring 5.00 x 11.00 m with 6 replications and have not use experimental design. Each plot have 6 line with 25 plant in row. Sweet corn was used Sugar 75 S&G. Infection of pathogen occurs naturally, and tested by Koch's postulates on maize age of 8 days. Disease severity measure every 7 days, from 21 day after planting (dap) until 63 day used Pataky method (Pataky et al., 2002). Disease incidence measure 5 times, from 21 dap until 49 dap. Model of disease progression were tested with three theoretical models: Logistics, Monomolekuler, and Exponential. These models have been corrected by Neher and Campbell 1992 (Rice and Pope, 2006; Rivai, 2001) models of disease progression is determined by the largest of value of the coefficient of determination ($R^2$), and the smallest of mean squares residual (MSR). The spread pattern of the disease were used Run analysis and corrected by Z test. The data were processed using SPSS version 17.

## 3. Result and Discussion

1. Disease Progress of Stewart's Wilt

Disease severity of Stewart's wilt in sweet corn plants in the field at 63 days after planting (dap) for 6 replications can be seen in Table 1.

Result of testing from 3 models of disease progression (Logistic, Monomoleculer, and Eksponensial) of Stewart's wilt in the field based on the value of $R^2$ and MSR, can be seen in Table 2.

Table 1. Disease severity of Stewart's wilt on sweet corn plants in the field (63 dap)

| Replication | Disease severity | |
|---|---|---|
| | % | rate |
| I | 12.22 | 0.27 |
| II | 15.70 | 0.32 |
| III | 6.89 | 0.14 |
| IV | 23.63 | 0.55 |
| V | 10.88 | 0.24 |
| VI | 22.37 | 0.52 |
| Everage | 15.28 | 0.34 |

Table 2. Model of disease progression of Stewart's wilt in the field based on the value of $R^2$ and MSR.

| Replication | Model | $R^2$ | MSR | Result |
|---|---|---|---|---|
| I | Logistik | 85.2 | 0.165 | |
| | Monomolekuler | 99.2 | 0.000 | Monomolekuler |
| | Eksponensial | 84.0 | 0.163 | |
| II | Logistik | 85.6 | 0.105 | |
| | Monomolekuler | 98.3 | 0.000 | Monomolekuler |
| | Eksponensial | 84.2 | 0.101 | |
| III | Logistik | 91.6 | 0.044 | |
| | Monomolekuler | 99.8 | 0.000 | Monomolekuler |
| | Eksponensial | 91.2 | 0.044 | |
| IV | Logistik | 91.5 | 0.104 | |
| | Monomolekuler | 98.3 | 0.000 | Monomolekuler |
| | Eksponensial | 89.9 | 0.102 | |
| V | Logistik | 88.0 | 0.087 | |
| | Monomolekuler | 97.2 | 0.000 | Monomolekuler |
| | Eksponensial | 87.2 | 0.083 | |
| VI | Logistik | 69.2 | 0.067 | |
| | Monomolekuler | 99.3 | 0.000 | Monomolekuler |
| | Eksponensial | 66.6 | 0.053 | |

Model of disease progress is Monomolekuler. This result is consistent with research of Morales et al. (2003) on spatial-temporal analysis of Stewart's wilt disease on corn in Mexico, he got a model Monomolekuler more fit than the model Logistics, Exponential, and Gompertz to corn varieties Triunfo (incidence of 26.13%) and varieties 9Bx52 (incidence 26.87 %).

Arias et al. (2013) get a model Monomolekuler too on the development of the disease charcoal root rot (CRR) in the pine trees caused *Macrophomina phaseolina*, that model suits both the greenhouse and field trials than Logistic and Gompertz.

Monomolekuler model ussually found on pathogens that are monocyclic. According to Rivai (2005) characteristic of the Monomolekuler model is assumption that the disease at plants can not directly cause other plants sick. Monomolekuler model assumes that the maximum level of disease is one, so that the disease severity or incidence of the disease is a diseased plant tissue proportion ranged from 0 - 1 (healthy - sick).

Carvalho et al. (2013) got the Monomolekuler model is the most suitable model for stem bleeding incidence (resinesis) on coconut plants caused by *Thielaviopsis paradoxa* in Sergipe, Brazil. Likewise Cammann et al. (1995) got the Monomolekuler model too on each cultivar to disease topovirus tomato spotted wilt (TSWV). Data and analysis are consistent with the hypothesis, where most infections appear as a result of the primary transmission then further spread of infection through secondary after TSWV virus survive in the land.

The results of this study differ from the results of Liu (2010) to the model of disease progress of *P. stewartii* subsp. *stewartii* in the plant, where he gained Exponential models. The occurrence of different models between our research because Liu research (Liu, 2010) had a model of pathogen progress while our research had a model of disease progress.

According to Campbell and Madden (1989), the results of measurements of disease severity is closely related to several things, among others: the method of measurement used, the researchers who conducted the observations, and circumstances where ongoing research. Added by Burbank (2014), during the process of infection, *P. stewartii* subsp. *stewartii* have to contend with the pressures that occur during the ongoing changes in the environment, such as the production of reactive oxygen species (ROS) in the host, and nutrient limitation.

The results of the study Godoy et al. (2003) had the model curve progression *Southern rust* in maize in Brazil is the Logistics better suited than the Gompertz, with $R^2$ values ranging from 0.78 to 0.99, where the research took place in two different geographic areas for two years. While Aghajani et al. (2010) had the disease progress model of *Sclerotinia stem rot* in Canola plants in Iran is Gompertz based on the value of $R^2$ (94.69%) with disease progression between 0.003 to 0.077.

Analysis of the Plant and Pest Diagnostic Laboratory at Purdue University (2012), Stewart's wilt suited to areas where the content of phosphorus and nitrogen is high, there are beetles flea, maize takes place in April-June, and air temperatures and high soil moisture, Likewise, the results of research of Aydogdu and Boyraz (2011) which conducts research on the effect of nitrogen and organic fertilizers on the severity of the disease of corn smut (corn smut (DC) Corda) and prove that the severity of the disease has increased due to the provision of nitrogen and organic fertilizers, so the amount of nitrogen and organic fertilizer is an important factor affecting the severity of the disease.

Likewise with the opinion of Dutta et al. (2011) who used regression techniques to combination of rainfall and minimum temperatures, and proves an increase rate of the disease by 98.4 % and can be used as a predictor. So rainfall and the minimum temperature is the variable weather that contributed most to the increase in the rate of black rot (*Xanthomonas campestris*) on chilli plants. According to Mina and Sinha, 2008 in Lopez et al. (2012), with a temperature increase sufficient soil moisture allows increased evapotranspiration so that the moisture microclimate around the plant can cause pathogens appropriate under the circumstances.

## 2. Spread Pattern of Stewart's Wilt Disease

Disease incidence of Stewart's wilt in sweet corn plants in the field at 63 days after planting (dap) for 6 replications can be seen in Table 3.

Standard value on the Z test is -1.64. If the value of $Z < -1.64$ then the dispersal patterns belonging to the aggregate pattern, on the contrary, if the value of $Z > -1.64$ the dispersal patterns classified in a random pattern (Campbell and Madden, 1989). Based on the Z test, the spread pattern of Stewart's wilt is Random (Table 4).

Stewart's wilt disease spread pattern is Random. These results are similar to the spread pattern of Stewart's wilt disease in the field obtained by Liu in Iowa, according to Liu (2010), the spread pattern of disease is not related to the percentage incidence or severity of the disease, but the spread of the disease is closely associated with the role of vectors that are likely to move to other plants.

Table 3. Disease incidence of Stewart's wilt on sweet corn plants in the field (63 dap).

| Replication | Disease incidence | |
|---|---|---|
| | % | rate |
| I | 23.33 | 0.76 |
| II | 28.00 | 0.81 |
| III | 22.00 | 0.73 |
| IV | 40.67 | 1.44 |
| V | 28.00 | 0.93 |
| VI | 28.67 | 0.85 |

| | | |
|---|---|---|
| Everage | 28.45 | 0.93 |

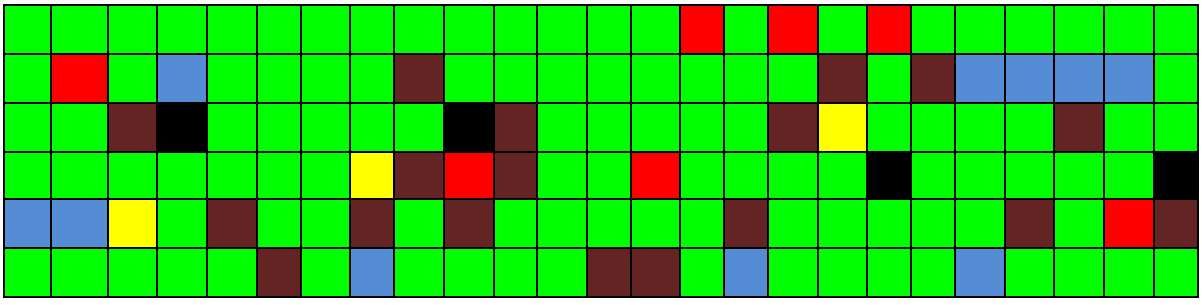
Table 4. Spread patterns of Stewart's wilt on sweet corn.

| Replication | Run U | Expected Run E(U) | Variances(U) | Z | Result |
|---|---|---|---|---|---|
| I | 54,00 | 54,67 | 4,36 | - 0,15 | Random |
| II | 54,00 | 62,35 | 5,37 | -1,55 | Random |
| III | 47,00 | 51,35 | 3,96 | -1,10 | Random |
| IV | 68,00 | 73,39 | 5,89 | -0,92 | Random |
| V | 59,00 | 61,48 | 4,91 | -0,51 | Random |
| VI | 59,00 | 62,35 | 4,99 | -0,67 | Random |

Meanwhile, according to Gilligan, 1982 *in* Cammann et al. (1995), the spread or dispersal patterns of spatial plant diseases are influenced by variations in biotic and abiotic factors that play a role at the time, ranging from pathogen interactions with a host of individual to be the level of a community, like ecological processes that influence the spread and environmental selection between the host plant and insect vectors.

If the observed spread pattern of the Stewart's wilt disease have been fulfilled several requirements Random pattern as the opinion of Cliff and Ord, 1981; Pielou, 1977; and Upton and Pigleton, 1985 *in* Sparks et al. (2008), among others: (i) each of the plants in the field have the same opportunities (but small) to be infected, (ii) by knowing the location of an individual, does not mean it is also known the location of another individual, ( iii) individual is not affected and does not affect other individuals in any form, and (iv) each individual occupies a unit freely and randomly.

For the first spreat pattern of stem bleeding (resinensis) on coconut plant caused by *Thielaviopsis paradoxa* in Sergipe, Brazil had a random and later became aggregate. It is proved that the disease was originally derived from the structure survived the pathogen itself, followed by infection of the plant by humans, contact between the roots, or via vectors *Rhynchophorus palmarum* that spread from plant to plant (Carvalho et al., 2013).



Note :

= plant infected at 21 dap

= plant infected at 28 dap

= plant infected at 35 dap

= plant infected at 42 dap

= plant infected at 49 dap

Likewise, research of Azahar et al. (2011), on the spread pattern of basal stem rot (BSR) is random during the 4 years of observation on the density of the plant A, B, and C. Based on preliminary analysis, he concludes distribution BSR disease is not associated with the density of plants. The study also concluded that the spread of BSR disease incidence is much higher in dense plantation crops compared with less land crop density. The existence of BSR disease is not a function of the crop to the infection, but the disease pressure in plantation areas. While the analysis of Moran's autocorelasi used Byamukama et al. (2010) tested the spread pattern of bean pod mottle virus (BPMV), obtaining aggregate patterns among countries in Iowa, this proves that the incidence BPMV between countries was not random.

Likewise, Cammann et al. (1995) conducted a study of tomato spotted wilt topovirus (TSWV), significantly detected a aggregate pattern most samples, but continuously occur random patterns until close to the aggregate due to the spread of the virus is determined predominantly by the vector that immigrated and is an important aspect in the development of the epidemic.

## Conclusion

From research, we can accept: 1) Model of disease progress of Stewart's wilt on sweet corn is Monomolekuler, 2) Spreat patterns of Stewart's wilt on sweet corn is Random.

## References


Arias, S. G., Rafael, R. P., and Eugenio S.V.S. 2013. Temporal analysis of charcoal root rot in forest nurseries under different pathogen inoculum densities and soil moisture content.. Tropical Plant Pathology Brasilia, 3, …. - ….

Aghajani, M. A., Safaie, N. and Alizadeh, A. 2010. Disease progress curves of sclerotinia stem rot of canola epidemics in Golestan Province, Iran". Journal Agronomi Science Technology, 12, 471-478.

Aydogdu, M. and Boyraz, N. 2011. Effect of nitrogen dan organic fertilizer on corn smut (*Ustilago maydis* (DC) Corda). African Journal of Agricultural Research, 6, 4539-4543.

Azahar, T. M., Jawahir, C. M., Mazliham, S., and Patrice, B. 2011. Temporal analysis of basal stem rot disease in oil palm plantations: An analysis on peat soil". International Journal of Engineering & Technology IJET-IJENS, 11, 96-101.

Baylor Collage of Medicine. 2006. About *Pantoea stewartii. ……. ……*

Burbank, L. P. 2014. Global regulation of virulence determinants during plant colonization in the bacterial phytopathogen, *Pantoea stewartii* subsp. *Stewartii.* Disertation University of California. ……..

Byamukama, E., Robertson, A. E., and Nutter, F. W., Jr. 2010. Quantification of temporal dan spatial l dynamics of *Bean pod mottle virus* at different spatial scales". Plant Health Progress, ...., .... - ...

Cammann, M. A., Culbreath, A. K., Pickering, J., Todd, J. W. and Demki, J. W. 1995. Spatial temporal patterns of spotted wilt epidemic in peanut". Phytopathology, 85, 879-885.

Campbell, C. L., and Madden, L. V. 1989. Introduction to plant disease epidemiology. John Wiley & sons. New York.

Carvalho, R. E., Souza, P. E., Warwick, D. R. N., Pozza, E. A., and Carvalho Filho, J. L. S. 2013. Spatial dan temporal analysis of stem bleeding disease in coconut palm in the state of Sergipe, Brazil". Anais da Academia Brasileira de Ciências, 85, 1567-1576.

Coffman, C. G. 1998. Critical Growth Stages of Corn". Texas Agricultural Extention Service. ……

Desi, Y., Trimurti, H., Ujang, K., Agustian, Syamsuwirman, and Prima, N. 2014. Characteristics of morphology and physiology isolate *Pantoea stewartii* subsp . *stewartii* on maize". Jurnal Fitopatologi Indonesia, 10, …. - ….

Dutta, S., Thapa, G., Barman, A. R., Hembran, S. and Khatua, D. C. 2011. 2011. Prediction of black rot disease progression of cabbage based on weather parameters". Rajshahi University Journal of Environmental Science, 1, 35-39.

EPPO Buletin. 2006. Diagnostics *Pantoea stewartii* subsp. *Stewartii".* European dan Mediterranean Plant Protection Organization, 36, 111-115.

Esker, J. O. and Nutter, F. W. 2002. The temporal distribution of *Chaetocnema pulicaria* In Iowa". Journal of Economic Entomology, …., …. - ….

Garden Guide. 1997. *Growth Stages of Sweet Corn*". ……..

Godoy, C. V., Amorim, L., Filaho, A. B., Silva, H. P., Silva, W. J., and Berger, R. D. 2003. Temporal progress of southern rust in maize under different environmental conditions. Fitopatologia Brasileira, 28, …. - ….

Gray, M. 2008. Corn flea beetle : Expectation for injury in 2008". University of Illinois Extension. Urbana.

Gregory, L. V., Ayers, J. E., and Nelson, R. R. 1978. Predicting yield losses in corn from southern corn leaf blight. The American Phytopathological Society. St. Paul Minoseta.

Ministry of Agriculture RI. 2015. Attachment regulation of the Minister of Agriculture No. 51/Permentan/Kr.010/9/2015 corcerning amentments to the regulation of the Minister of Agriculture No. 93/Permentan/OT.140/12/2011 concerning type plant quarantine". ……

Liu, L. 2010. "Quantifying The Aggressiveness, Temporal and Spatial Spread of *Pantoea stewartii* In Sweet Corn". Thesis Iowa State University. Ames, Iowa.

Lopez, R. Y., Pacheco, I. T., Guevara-Gonzalez, R. G., Hernandez-Zul, M. I., Quijano-Carranza, J. A., and Rico-Garcia, E. 2012. ..... African Journal of Biotechnology, 11, 2417-2428.

Morales, G., Silva, H. V., Ochoa, D. L., Carballo, A., and Cordova, L. 2003. Spatio-temporal analysis of Stewart's wilt of corn caused by *Pantoea stewartii* in Mexico". Phytopathology, 94, S72.

Munkvold, G. P. and Rice, M. 1997. Stewart's disease risk for 1997. Integrated Crop Management (ICM) IC-478(8). Iowa State University.

Munkvold, G. P. 2001. Corn Stewart's Disease". University Extension. Iowa States University. ……

Pataky, J. 2000. Stewart's wilt of corn. Department of Crop Sciences. University of Illinois. Urbana.

Pataky, J. K., du Toid, L. J., and Freeman, N. D. 2002. Stewart's wilt reaction of an international collection of *Zea mays* germ plasm inoculated with *Erwinia stewartii"*. Plant Disease, 8, 901-906.

Pataky, J. K. and Ikin, R. 2003. Pest risk analysis, the risk of introducing *Erwinia stewartii* in maize seed". International Seed Federation. 1260 Nyon, Switzerland.

Pataky, J. K., Michener, P. M. 2004. Ability of an ELISA-based seed health test to detect *Erwinia stewartii* in maize seed treated with fungicides dan insecticides". The American Phytopathology Society. ……..

Plant and Pest Diagnostic Laboratory. 2012. Purdue University ……..

Pollock, C. 2002. Stewart's bacterial leaf blight predicted severe this year". Ohio States University. ……

Rice, M. E., dan Pope, R. 2006. Predictions for corn flea beetles dan Stewart's Disease in 2006". Integrated Crop Management (IPM). IC-496(7). Iowa State University. ……..

Rivai, F. 2001. Plant Disease Epidemiology. Universitas Andalas. Padang.

…… 2005. Basics of Plant Disease Epidemiology. Yayasan Perguruan Tinggi Komputer UPI PRESS. Padang.

…… 2006. Yield Loss Cause Plant Disease. Andalas University Press. Padang.

..... 2009. Spatial and Temporal in Plant Disease. Universitas Baiturrahmah. Padang.

Sparks, A. H, Esker, P. D., Bates, M., Acqua, W. D, Guo, Z., Segovia, V., Silwal, S. D., Tolos, S., and Garnett, K. A. 2008. Ecology and epidemiology in R : Disease progress over time. The Plant Health Instructor. …….. …….

Subekti, N. A., Syafruddin, Roy, E., and Sri S. 2003. Plant morfology and growth phase of maize". Balai Penelitian Tanaman Serealia. Maros ……..

Xu, R., Chen, Q., Djama, Z. R., and Tambong, J. T. 2009. Miniprimer PCR assay targeting multiple genes: A new rapid and reliable tool for genotyping *Pantoea stewartii* subsp. *Stewartii"*. …….. Canada.

Yang, X.B. 2003. Risk Assessment: Concepts, Development, and Future Opportunities". Plant Management Network. Iowa State University. …….

# On growth response and results of upland rice due to the allotment of some a dose of compost bamboo leaves

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## Abstract

The research "On Growth Response and the Results of Upland Rice due to the Allotment of Some a Dose of Compost Bamboo Leaves". On the field and laboratory of Agriculture Faculty, Muhammadiyah University of West Sumatra. Research purposes, to get a dose of compost bamboo leaves which is proper for upland rice . This is a experiment in Completely Random Blok Design,, with 5 level and 3 replication of dose of compost bamboo leaves 0 , 5 , 10 , 15 and 20 t / ha. Data observation analysed statistically by test f the first real 5 percent. The results showed research for growth and uplang rice the results of a dose 20 t / ha give the best results for growth and the results of upland

Keywords: Compost bamboo leaves, upland rice, ……..

## 1. Introduction

Indonesia today face food problems resulting from the rise in the number of people who followed many rice fields fertile ber irigasi, shift function to become an industrial area and settlement .In addition of the recent disasters in the form of long dry season flood and almost every year , so as to meet their governments national import rice reached 1.428.505,678 t and us score $ 291.422.862 (BPS, 2003a), hence challenges ahead is how improving the results of rice farming and rice gogo .

BPS in 2004 reported that the average productivity rice gogo in indonesia only reached 2.66 t $ha^{-1}$, with the area under harvest 1.04 million ha and contributing only 5.01 % of the results of rice national. The average yield is still very low because rice gogo commonly planted marginal soil and use conventional system (Soeraptoharjo and Suwarjo, 1988). One effort to increase production rice gogo in the use of compost dregs leaves bamboo.

Research purposes to get compost bamboo doses leaves the best so can increase growth and upland rice results.

## 2. Material and Methods

The research is field testing , material used in experiments is: varieties of upland rice Situ Bagendit there , fertilizer Urea , SP-36 and KCl (200 kg $ha^{-1}$ Urea, 250 kg $ha^{-1}$ SP-36 and 100 kg $ha^{-1}$ KCl . Compost leaves bamboo, granting compost about 0, 5, 10, 15 and 20 t / ha in give a week before planting. Fertilization inorganic in give doses urea while SP- 36 and KCl entirely, when cropping then doses urea age 40 days. Care done watering otherwise rain .Weeding weeds done manually to revoke weeds at the time of 2 WAP and 6 WAP, while pest and disease control done wisely.

The research uses method experiment used is a random a group (a shelf) with 5 treatment and 3 replication. All the data observation obtained analyzed by test f the first real 5 percent, if markedly dissimilar followed by Duncan s New Multiple Range Test (DMRT) the first real 5 percent.

Observation is observation tall plant, the number of saplings per a thicket, the percentage saplings productive per a thicket, the number of panicles per a thicket, long panicles, the number of grain per panicles, dry weight grain per a thicket or ha and weights 1000 seeds.

## 3. Result and Discussion

3.1. Plant height (cm).

The average tall plant rice after tested said DNMRT the first real 5 % can be seen in Table 1.

Table 1. Tall plant rice on some doses compost leaves bamboo age 10 mst.

| Doses compost leaves bamboo (t/ha) | Plant height (cm) |
|---|---|
| 0 | 96.31 |
| 5 | 100.15 |
| 10 | 102.39 |
| 15 | 103.57 |
| 20 | 105.00 |
| KK = 3.51 % | |

Numbers at the column followed by the same letters is not significantly different at DMNRT with 5% level of probability.

Table 2. Total plants per bunch per bunch of paddy on various doses compost leaves bamboo age 10 WAP.

| Doses compost leaves bamboo (t/ha) | Total plants per bunch (stems ) | |
|---|---|---|
| 0 | 16.31 | a |
| 5 | 20.15 | a |
| 10 | 21.39 | a |
| 15 | 24.57 | b |
| 20 | 30.01 | c |
| KK = 22.12 % | | |

Numbers at the column followed by the same letters is not significantly different at DMNRT with 5% level of probability.

Table 3. Percentage of productive paddy plant at of some doses compost leaves bamboo age 10 WAP.

| Doses compost leaves bamboo (t/ha) | Percentage of productive (%) |
|---|---|
| 0 | 76.31 |
| 5 | 78.15 |
| 10 | 79.39 |
| 15 | 80.57 |
| 20 | 81.35 |
| KK = 2.53 % | |

Numbers at the column followed by the same letters is not significantly different at DMNRT with 5% level of probability.

Table 4. Total panicles of paddy plant on various doses compost leaves bamboo age 10 WAP

| Doses compost leaves bamboo (t/ha) | Total panicles per bunch (bunch) | |
|---|---|---|
| 0 | 10.31 | a |
| 5 | 12.15 | a |
| 10 | 15.39 | a |
| 15 | 22.57 | b |
| 20 | 23.10 | b |
| KK = 12.15 % | | |

Table 5. Longest panicles length of paddy plant on various doses compost leaves bamboo age 10 WAP.

| Doses compost leaves bamboo (t/ha) | Longest panicles (cm) |
|---|---|
| 0 | 19.31 |
| 5 | 20.15 |
| 10 | 21.39 |
| 15 | 22.57 |
| 20 | 23.98 |

| KK = 2.61 % |
|---|

Numbers at the column followed by the same letters is not significantly different at DMNRT with 5% level of probability.

From Table 1, can be seen that high rice plants showed no real different his neighbor either between the influence of various dosages compost bamboo leaves. It is suspected that this tall plant influenced the nature of genetic of the plant itself. In accordance with statements from Gadner, Pearce and Mitchell (1991) that plants affected by genetic including higher plants.

To research Yusnaweti in 2014 of upland rice varieties Lake Gaung has also in be high is no different real ter turn to several species of fungi mycorrhizal arbuskula.

3.2. Total plants per bunch (stems ).

The average total plants per bunch of paddy plant after further tested with DNMRT at 5% level of probability were shown in Table 2.

In table 2, can be seen that total plants per bunchs per bunch of paddy doses compost leaves bamboo 20 t / ha shows the number of saplings per a thicket highest namely 40.01 a distinct stem with real disis compost 15, 10, 5 and 0 t / ha. But between doses compost leaves bamboo 10, 5 and 0 t / ha not markedly dissimilar his neighbor.

It is suspected that this that in doses compost leaves bamboo 20 t / ha had instances of hara more widely available and providing to the growth of plants .The results of the study Agustamar in 2007 levels hara compost Tithonia ( BO = 50.49 % , C-org = 29.28 % , C/N = 9.27 , N = 3.16 % , P = 0.73 % , K = 3.97 % and the water level = 17.91 % and compost beef ( B.O = 35.45 % , C-org = 20.56 % , C/N = 13.39 , N = 1.54 % , P = 0.43 % , K = 1.57 % and the water level = 37.06 % ) . Hara enough to be increase photosynthesis that will produce the number of saplings per of more

3.3. Percentage of productive plant per bunch (%)

The average percentages of productive plant per bunch of paddy plant after further tested with DNMRT at 5% level of probability were shown in Table 3.

In Table 3, can be seen that the puppies productive of paddy show non significant differences fellow various compost leaves bamboo doses.This might have been caused the percentage of saplings productive is the result comparison all the children with a total produce panicles puppies formed at times 100 %, here is where all plants and produce panicles is directly proportional to total saplings formed caused the results of the percentage of saplings productive show no same unreal because in this research using a kind of varieties of rice echo gogo that is the sea. Varieties determine long growth puppies and the number of puppies rumpun$^{-1}$. These findings Defeng et al. (2002) that the number of puppies productive highly influenced by varieties are used.

3.4. Total panicles per bunch

The average total panicles per bunch after further tested with DNMRT at 5% level of probability were shown in Table 4.

Numbers at the column followed by the same letters is not significantly different at DMNRT with 5% level of probability.

In Table 4 , can be seen that that the number of panicles rice gogo given various doses compost leaves bamboo on the highest doses 20 t / ha namely 23.10 cm is no different real with 15 t / ha and markedly dissimilar with 10 , 5 and 0 t / ha .This supposedly associated with the more compost who ddiberikan so element hara the more available so that the number of panicles in doses compost leaves bamboo 20 t / ha the most. This is in accordance with the results of the study Gusnidar in 2007 that the number of panicles will be increased a lot as increased

doses compost tithonia given on rice farming intensification

3.5. Longest panicles (cm)

The average of longest panicles of paddy plant after further tested with DNMRT at 5 % level of probability were shown in Table 5.

In Table 5, can be seen that long panicles of paddy does not disclose not same between doses compost leaves bamboo 0, 5, 10, 15 and 20 t / ha. It is suspected that this because in environmental conditions that quite beneficial as the water supply, hara and light the sun will to get the growth of plants normal, so long panicles formed only determined by genetic factors namely varieties of a plant. On trial it uses is the same namely varieties there Situ Bagendit, so long panicles produced the same. This is in accordance with opinion Gadner, Pearce and Mitchell ( 1991 ) that plants influenced by genetic including long panicles.

3.6. Total grains per panicles (grains)

The average total grains per panicles of paddy plant after further tested with DNMRT at 5% level of probability were shown in Table 6.

In Table 6, can be seen that the number of grain per panicles upland rice who were given compost leaves bamboo show had been a clear sesamanya. Next to doses compost leaves bamboo 20 t / ha rice gogo show higher namely 178.50 the different with real doses compost bamboo leaves 15, 10, 5 and 0 t / ha.

Table 6. Total grains per panicles of paddy plant of paddy on various doses compost leaves bamboo

| Doses compost leaves bamboo t/ha) | Total grains per panicles (grains) |
|---|---|
| 0 | 110.31 a |
| 5 | 120.15 a |
| 10 | 123.39 a |
| 15 | 144.57 b |
| 20 | 178.50 c |
| KK = 20.67 % | |

Numbers at the column followed by the same letters is not significantly different at DMNRT with 5% level of probability.

Table 7. Grains weight per panicles of paddy on various doses compost leaves bamboo.

| Doses compost leaves bamboo (t/ha) | Grains weight Per panicles (g) t/ha |
|---|---|
| 0 | 10.11 a |
| 5 | 2.52 a |
| 10 | 10.15 a |
| 15 | 2.53 a |
| 20 | 11.00 a |
| | 2.75 a |
| | 12.27 b |
| | 3.01 b |
| | 15.52 c |
| | 3.85 c |
| KK = 12.31 % | |

Numbers at the column followed by the same letters is not significantly different at DMNRT with 5% level of probability.

This might have been caused the number of grain per panicles derived from total panicles and long panicles and in fact that the number of grain per panicles positively correlate very real by the number of panicles and long panicles which means the number of panicles and long panicles significantly very determining the amount of grain per panicles. On the number of ample panicles and long panicles long will produce sum grain many.

The results of the study Agustamar, Ahmad and Sondang, (2012) fertilizer compost the organo of complex is fertilizer organic that can fix the structure of the soil and the land be friable and roots can develop well and Agustamar in 2007 levels hara compost tithonia ( B.O = 50.49 % , C-org = 29.28 % , C/N = 9.27 , N = 3.16 % , P = 0.73 % , K = 3.97 % and the water level = 17.91 %

) while in compost beef ( B.O = 35.45 % , C-org = 20.56 % , C/N = 13.39 , N = 1.54 % , P = 0.43 % , K = 1.57 % and the water level = 37.06 % ).

Hara enough to be increase photosynthesis that will produce production dry substances more the number of grain per panicles of paddy does not disclose a clear different his neighbor either between the influence of various doses compost.

3.7. Grains weight per panicles (g) and t/ha

The average grains weight per panicles of paddy plant after further tested with DNMRT at 5% level of probability were shown in Table 7.

In Table 7, can be seen that weight dry grain per a clump or per hectare of paddy show the provision of doses compost leaves bamboo 20 t / ha showed weight dry grain per a clump or per hectare higher doses different with real 15 , 10 , 5 and 0 t / ha .It is suspected that this hara available was much so sufficient to the growth of plants.

Research Gusnidar in 2007 the Tithonia 7.5 t $ha^{-1}$ in rice farming improve the result grain of 20.51 - 21.08 g $pot^{-1}$ ( 18.69 - 19.21 % ) .The results of the study judges and Agustian ( 2003: 2004: 2005 ) , the provision of tithonia dry as much as 4 t $ha^{-1}$ ( 24 t $ha^{-1}$ tithonia fresh together to mensubtitusi 50 % needs chemical fertilizer ) on the ground ultisol for plants chili and ginger the results chili as many as 9.36 t $ha^{-1}$ and ginger fresh as many as 11 t $ha^{-1}$.

3.8. Weight of 1000 grains (g)

The average weights of 100 grains of paddy plant after further tested with DNMRT at 5% level of probability were shown in Table 8. In Table 8, can be seen that of paddy does not disclose a clear different neighbor either between the influence of various doses compost between 0 , 5 , 10 , 15 and 20 t / ha. It is suspected that this for using varieties same namely verietas there bagendit showed the similarity variations in the quantity of cells and cell size seeds, so here more of a role genetic trait of a plant, although different treatment but treatment has yet to significant not can change environment growing so that it will remain give weight 1000 seeds almost the same.

Table 8. Weight of 100 grains of paddy on various doses compost leaves bamboo

| Doses compost leaves bamboo (t/ha) | Weight of 1000 grains (g) |
|---|---|
| 0 | 26.31 |
| 5 | 26.95 |
| 10 | 27.39 |
| 15 | 27.45 |
| 20 | 27.50 |
| KK = 2.23 % | |

Numbers at the column followed by the same letters is not significantly different at DMNRT with 5% level of probability

According to research Yusnaweti in 2015 also get the number of grain 1000 seeds not markedly dissimilar on variety Danau Gaung. The weighting of 1000 seeds that uses green manure tithonia and manure with various distance cropping on rice varieties beautiful gogo also shows the weighting of 1000 seeds that is not markedly dissimilar (Bilman, 2008)

## Conclusion

Virtue of the outcome of the experiment response growth and the results of rice gogo due to the provision of a couple doses compost leaves bamboo it turns out that the provision of doses 20 t / ha can provide growth and the best results in upland rice.

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## References

Gardner, F. P., Pearce, R. B. , and Mitchell, R. L. 1991. Physiology cultivated plants. Publisher University of Indonesia . 428 thing.

Soepraptohardjo and Suwardjo, H. 1988. Land and potential land for rice. In rice books 1. The Agricultural Research and Development. The Central Research and Development food crops. Bogor. It 271-293.

The Central Bureau of Statistics. 2003a. Statistics foreign trade Indonesia, import 2003. The central Bureau of Statistics. Jakarta. Indonesia

The Central bureau of Statistics. 2004. Statistics Indonesia. Jakarta.

Agustamar, bs.Ahmad and he.Sondang. 2011. Design formulation the organ of complex in-siu to assembly technology sri (the Ssystem of Rice Intensification in rice fields openings new.Report peneliian hiber th 1. Polytechnic agricultural land, Payakumbuh

Agustamar , B. S . Ahmad and Sondang, Y. .2012. The Design of complex formulation of the organ in-siu to assembly technology SRI ( the System of Rice Intensification) on rice fields new openings. Reports peneliian hiber th 2. Polytechnic Payakumbuh Aagricultural land.

Yusnaweti. 2015. Respon of different species Arbuscuular Mycorrhizae fungi and adoption sri method on the growth and production od upland rice on ultisol

Defeng, Z., Shihua, C., Yuping, Z., and Xiaqing, L. 2002. Tillering patterns and the contribution of tillers to gain yield with hybrid rice and wide spacing. China National Rice Reasearch Institute, Hangzhou. CIIFAD, http://ciifad.cornell.edu/sri:ciifat@cornell.edu. 125-131 p.

Gusnidar. 2007. Cultivation and use of Tithonia diversifolia to save fertilization N, P and K rice field laborers intensification. Dissertation. The Program Univ Graduate. Andalas. Padang. The. 256 it.

Bilman. 2008. A modification of the environment through the system as well as the addition of planting organic material controller and substance grow in efforts to increase rice productivity gogo ( *Oryza sativa L.*). Dissertation. Successor Program Graduate. Andalas University . Padang. 210 thing

Yusnaweti. 2014. Response rice gogo to several kinds of organic matter and fungi *Mycorrhizal arbuskula* in order to improve ultisol outcomes Dissertation.The Program Graduate Andalas University . Padang. The.184 it.

# Rice husk biochar application in traditional paddy soil and its effect of nutrients vertical distribution

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## Abstract

A research to investigate nutrients vertical distribution in traditional paddy soil system after several doses appliction of rice hulk biochar, was conducted from May to August 2015 atTanjung Betung Village, Pasaman District, Province of Sumatera Barat, Indonesia.The experiment was designed on Completely Randomized Design (CRD) with 6 treatments and 3 replications. The treatments consist of A= 0 ton/ha, B= 5 ton/ ha, C= 10 ton/ha, D= 15 ton/ha, E= 20 ton/ha and F= 25 ton/ha. Soil samples were collected using soil argerbythe depth of 0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm and 40-50 cm. Soil and plant analysis carried out in the Laboratory of Soil, Faculty of Agriculture, Andalas University, Padang. Data from the analysiswere statistically tested with T test at 5% level. The results showed that rice hulkbiocharapplication influence the soil properties as well as the vertical distribution of soil nutrients. Biochar with a rate of 25 ton/ha in top soil were able to increase organic C at 27.98 ton/ha; total N at 14.45 ton/ha, $P_20_5$at 53.80 ton/ha; and $SiO_2$at 0.32 ton/ha. Soil profile nutrients after treatment with 25 ton/ha are, organic Cat 67.11 ton/ha; total N at 16.18 ton/ha;$P_20_5$at53.80 ton/ha;and $SiO_2$at 1.62 ton/ha. It can be assumed that the application of biochar enhance soil fertility and rice production in paddy soil.

Keywords : rice husks biochar, traditional paddy soil, Pasaman

## 1. Introduction

In the early 1960s, Indonesian government took action on achieving rice self-sufficiency extension known as Green Revolution (GR) which cointains mass guidance programs (bimas) and mass intensification program (inmas). Both of the programs supported each other in delivering technical guidance on rice fieldscultivation to farmers in rural areas. The term rice field as known as sawah refers to a leveled and bounded rice field with an inlet and outlet for irrigationand drainage (Wakatsuki et al., 1998). GR trend is an adoption of modern agricultural technologies, including irrigation, pesticides, and heavy doses of chemical fertilizer. Despite offering dramatic production gains, the GR technology surrounded by environmental problems. Runoff and leaching of fertilizer, pesticide and herbicide continue to be significant causes of environmental pollution, killing beneficial soil microbes and other organism; erosion of the soil; loss of valuable trace elements.

Implementation of GR technology caused a lot of changes in rice cultivation until a few years after. Eventhoughmodern

rice fields management has been growing rapidly, but in Pasaman Province of West Sumatra, Indonesia, there was a local seedfarm planted with a traditional ways. Darmawan et al. (2006) reported that natives community in Tanjung Betung village,Pasaman district prevalently using a traditional cultivation systems. Farmers of this region are notaffected by modernization of agriculture, both in terms of mechanization, the use of quality seeds, pesticides, and others.Wetland areas in TanjungBetungvillage, Indonesia is 423.5 ha. However, about266 ha irrigated with simple irrigation hold independently by local farmer communities.

As a consequents of traditional cultivation system, the depth of topsoil is less than 10 cm and bulk density values only about 1.6 g $cm^{-3}$ (Adly, 2015). The deterioration of soil organic matter (SOM) due to an imbalance input with decomposition rate might also be another effect responding to the less top soil. Meanwhile, a research by Safitri (2015) mentioned that top soil in traditional rice fields (TRF) in Tanjung Betung village,has a low SOMwhich only 9.4 g/kg. The dominance of aerobic situation in sawah soil caused decomposition of SOM running quickly. This is followed by cultivation activities carried out 1 to 2 times a year with intensive traditional management. The productivity of seedfarmshow a low amount due to the land management and conditions. Data from Safitri (2015) also showed thatthe value of production (yield/unit of land) inTRF only achieved at the rate of 2.5 ton $ha^{-1}$.

Meanwhile, soil organic carbon depletion increased emissionof greenhouse gases, and global warming are major concerns nowadays. Annual total $CO_2$equivalent emissions from world agriculture in 2011 were estimated to be 5.1–6.1 Pg and comprised about10–12% of global anthropogenic emissions (Smith et al., 2011). One of the most recent measures used to enhance the carbon sequestration in soils is addition of biochar. Biochar is produced through a pyrolysis process, when tissues of biological origin are burned or charred in the absence of, or at low levels, of oxygen (Lehmann et al., 2007). Conversion of wastes paddy harvest such as rice husk can be turn into biocharthrough pyrolysisand resulting advantages such as sustainable waste recycling, improvement of soil quality, and better plant growth.

Biochar has been shown to improve chemical, physical, and biological properties of soils (Yamato et al., 2006) and enhance plant growth (Ball et al., 2012). Rice husksbiochar contains organic carbon>35% and the content of macro nutrients such as N, P and K were high enough, therefore, waste rice husk can be processed into biochar that can be returned to the ground as soil conditioner. According to Kishimoto (2009) biochar or charcoal is a solid material that is formed from the carbonization of biomass. Biochar also has a function as a large enough binder of carbon. Therefore, rice husk biochar become an effective yet efficient material to increase food security and soil fertility of ricefields areas that have low SOM and limited input of chemical fertilizers.

Furthermore, in TanjungBetung nutrients vertical distribution showed different patterns among soil nutrient. Research conducted by (Septiza, 2014) indicate that the transfer of vertical nutrients in traditional rice fieldsoccurred because the*plow plan* has not yet formed. The rarely of landuse intensity and the minimum tillage by farmers leading the slow formed of *plow plan*.Land management and traditional cultivation systemaffected sawah soil properties in TanjungBetung. The purpose of the present study was to evaluate the effect of rice husk biochar treatment in sawah soil vertical distribution. In addition, the study attemped to determine how biochar affected rice productivity in TanjungBetung village.

## 2. Material and Methods

**Description of the study area and soil characterictic**

The experimental field of paddy rice is located in Tanjung Betung village, Pasaman District, in the province of West Sumatra, Indonesia. The study area dan sampling sites lies in 00°28'49.9"N to 100º02'55.5"E. The paddy rice was planted by using minimum tillage and local paddy variety, which was seeded on April 6, 2015. There were 18 experimental plots and 6 different doses of rice husk biochar consist of A= 0 ton/ha, B= 5 ton/ ha, C= 10 ton/ha, D= 15 ton/ha, E= 20 ton/ha and F= 25 ton/ha. The studied soil is classified as mixed Alluvium, from InsepticolOrdo. Figures 1 show thesite plan study in where all seedbed paddy was plant and soil samples taken.

Traditional cultivation system in Tanjung Betungis a simple and friendly environmental agricultural model that maximize landin-situ potential so the production costs will be minimized. Local communities adopted the minimum tillage farming using what they called "tajak" (machete that shaped like hoe) and digging the soil only at0-7 cm depth. Crop residues such as straw are carried when farmers tilling the soil and used as material for "emergency dike" that allowed for ± 10 days to be used as a source of organic matter, they called it "pamasaman". Since soil prepared with minimum tillage system, the plow layer became hard and compact, so the farmers use a spiky wood or "martunjuk" which help them in transplanting.

Moreover, the irrigation systems only source from rainfall water, and thus limit rice output. During harvesting period, local farmers did not use agricultural machinery but it's done by mutual cooperation and the use of term "mardege", which means threshing paddy by stepped it and afterwards separate the empty rice husk. Local knowledge by local farmers is the enactment of a mutual corporation through blood relatives.The activity has been going on for generations in this area.

**Biochar Apllication and soil samples**

Rice planting will be done by soil minimum tillage system, which soil ismanually treated by *tajak*. Subsequently, rice huskbiochar spread evenly over the soil surface in accordance by unamended soil (control=A) and amendedsoil by biochar that is B= 5 ton/ ha, C= 10 ton/ha, D= 15 ton/ha, E= 20 ton/ha and F= 25 ton/ha. Rice husk biochar spread evenly based on the treatment doses and combine with topsoil, sobiochar and soil will be mixed. Within application of basic ferlilizer such as N, P, K the rice fields ready to planted. Plants were harvested on day 90 when it had reached full maturity. Soil samples were collected using soil arger bythe depth of 0-10 cm, until 50 cm.

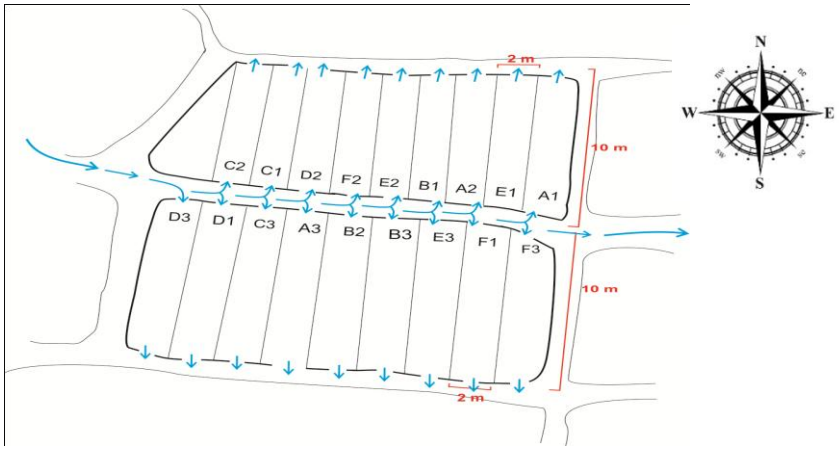


Figure 1. Research plots and soil sampling site at once in Tanjung Betung.

**Laboratory analyses**

*Chemical properties of soils*

Air-dried soil samples were ground and passed through a 2 mm sieve and stored in plastic boxes for laboratory analyses. Finely ground soil samples were oven dried at 105$^{o}$ C for approximately 48 h. Soil analyses such as organic Carbon using Wakley and Black method, total Nitrogen using Kjeldahl method, Pavaiable using Bray No. 2 method and Siavaiable with Pyrophosphate extract.

*Bulk density*

Bulk density is necessary for compared organic C transtitionbecaused both have an integral value. The bulk density of soil was calculated using the sample in a 100 cm$^3$ core. After being ovendried at 105$^{o}$ C for approximately 48 h, the weight of soil per core sample volum (100 cm$^3$) was measured.

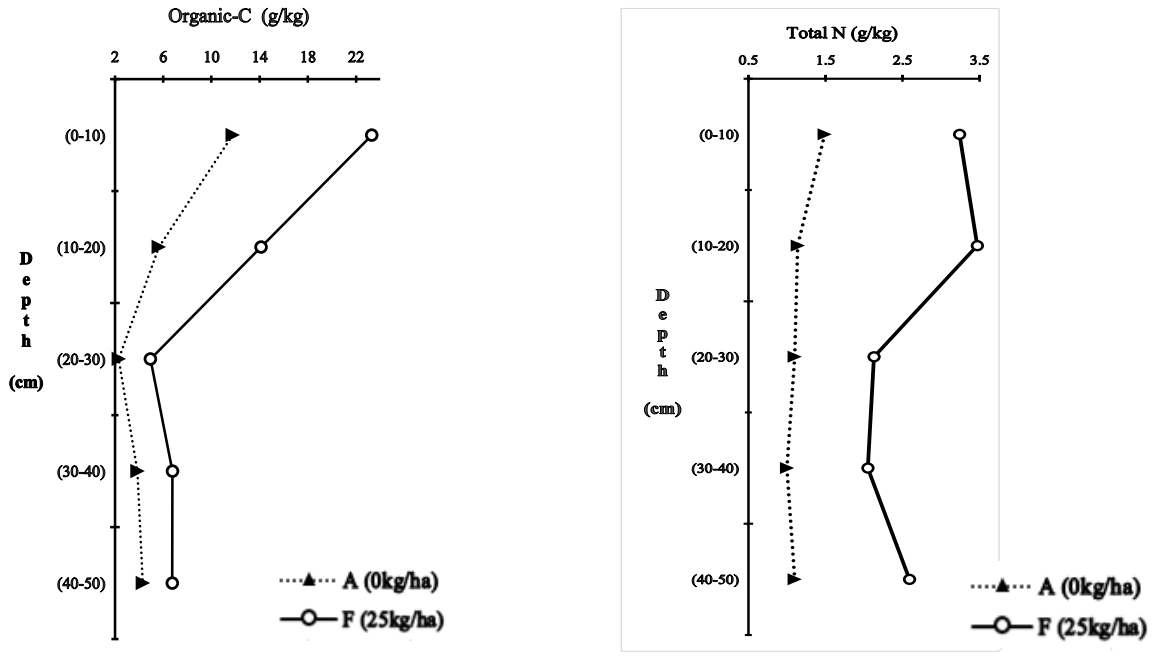
**Statistical analysis**

Microsoft Excel 2013for Windows 8 was used to statiscally examine the effect of biochar in paddy soil vertical distribution. To examine the relationship among soil properties, Perason's coefficient correlation was calculated.

## 3. Result and Discussion

**Nutrients vertical distribution under different doses of rice husk biochar**

Figure 2 shows the vertical distribution of organic C and total Nitrogen contents in paddy soil after rice husk biochar application. It can can be noted that biocharallocationhave the ability to increase the content of organic C in the topsoil 0-10 cm. At this depth seen that the highest content of organic C was found in F treatment and also increased the highest when compared with the control, A= (11.76 g/kg) and F= (23.30 g/kg) an increase of 98.13%.



**Figures 2**. Vertical distribution of the organic C and total N content in paddy soil after biochar treatments.

According Sujana (2014), rice hull biochar has an organic-C content of 20.86%, so the addition of biochar to soil can increase carbon. (Chan and Xu, 2009 citVerheijen et al., 2010) adds that the total amount of carbon in the biochar ranges from 172-905 g/kg.

Occurrence of C-organic topsoil followed by the declining value of land BV resulting improvement paddy soil physical properties. The C-organic inversely proportional to the value of the land BV with the decline reached 20.52%.

This is due to their high content of organic matter, the soil is relatively loose and there exudate which increases the activity of microorganisms so that the state of the soil becomes loose and granulated (TRP increase).

According to Ball et al. (1999) land traditionally processed can increase the amount of macropores and this situation can be sustained in the long term. This is needed to support plant growth. Another factor causing the high C-organic fertilizer is the addition of the base at the time of processing the soil will affect the process of mineralization of nitrogen that helps to improve the content of C-organic paddy soil (topsoil).

Figure 2also shown the total N content of the soil at a depth of 0-10 cm increase of treatment A= (1.49 g/ kg) to treatment F= (3.24 g / kg). This proves that in addition to donating N, rice husk addition of biochar can improve soil particles to prevent the loss of N, so that it can be available to the plants. Provision of rice hull biochar can also increase the value of N-total land of the criteria of low to moderate. Chaerun (2008) suggested the use of biochar in paddy fields can increase the number of bacterial nitrogen fixation (nitro-zobacter) in soil especially around the roots of crops.

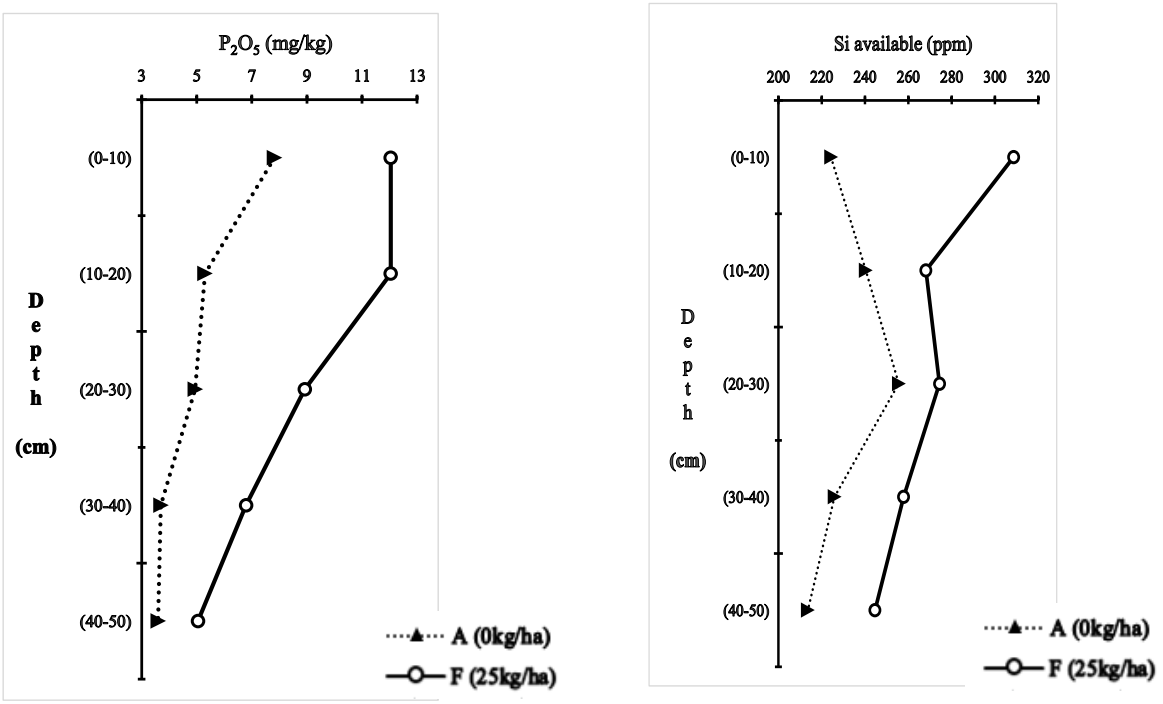
Increased of total N in soil subsoil is also suspected due to the provision of urea fertilizer during the three days before and thirty days after planting. Provision of inputs through fertilization is one source of N availability in the soil. Kyuma (2004) suggests the application of urea fertilizer in paddy fields affect the availability of soil organic matter helps to improve the content of N-total ground. While the rice hull biochar on any increase in dose, it also helps retain nutrients N, so that the N content increases. According to Gani (2009), when used as biochar soil amandement together with organic or inorganic fertilizer, biochar can increase productivity and nutrient availability to plants.

Provision of rice huskbiochar affect the value of P-available rice land more is shown in Figure 3. The increase in the value of P-provided occur from treatment A= (7.82 mg/kg) to treatment F= (12.04 mg/kg), while increasing by 54%. This indicates that administration of biochar very response to the availability of P. Biochar hold P not like other organic materials such as compost, green manure, manure, and others.

The content of available P decreased with increasing soil depth fields. The low availability of P with increasing depth is also expected due to lower soil pH. According to Kyuma (2004) the hydrolysis that occurs in the soil causes the P adsorbed at a lower pH. The availability of P in the soil under flooded affected by (a) the reduction of Fe3 + to Fe2 + -P -P, (b) the high solubility of Fe-P and Al-P in soil pH neutral, and (c) mineralization of organic phosphate. In addition, the low distribution of P on the bottom layer is also caused by a low addition of P fertilizer inputs by farmers over the years.

Si-available analysis results from application of biochar soil paddy rice husk is

shown in Table 8. In Figure 3, seen the value of Si-available at a depth of 0-10 cm of the lowest found in treatment A = (224.3 ppm), while the highest value contained in treatment E = (273.7 ppm). But the element of Si decreased in treatment D and E further rise in treatment F. Whereas at a depth of 10-20 cm linear increase in starts of treatment B.




**Figures 3**. Vertical distribution of the $P_2O_5$ and Si-available content in paddy soil after biochar treatments

From the analysis, Si available in soil is generally increasing at a depth of 20-30 cm up to 40-50 cm. This indicates that in addition to custom farmers use leftover rice straw as an emergency dike, allegedly material causes the accumulation of soil parent material Si increases with soil depth. According to Prasad et al. (2008) paddy soil derived from the parent material transported material (Alluvial) have high silica due to the influence of the type of clay mineral.

**Rice Productivity After Biochar Application**

Statistical analysis of the influence ofrice huskbiochar on the production is shown in Table 1. Table 1 shows that administration of rice husk biochar did not affect the production of paddy fields. Nevertheless, judging by the value, fixed an increase in production output in each addition of biochar dose.

Production of paddy in treatment A was lower, of 2.59 ± 0.44 tonnes / ha and continued to increase until the F-value

Table 1. Effect of ice husk biochar application in rice productivity.

| Treatments | Production (ton/ha) |
|---|---|
| A (0 ton/ha) | 2,59±0,44 |
| B (5 ton/ha) | 2,88±0,77 |
| C (10 ton/ha) | 3,05±0,29 |
| D (15 ton/ha) | 3,20±0,43 |
| E (20 ton/ha) | 3,29±0,39 |
| F (25 ton/ha) | 3,30±0,43 |

Noted: ±is Deviation Standard

treatments 3.30 ± 0.43 tonnes / ha. Despite the increase in production is not significant because the response of plants to the addition

of biochar is still low, but the biochar will release nutrients slowly so that the amount of nutrients contained in the soil also increased after several planting seasons.

Increased production of rice land allegedly because biochar rice husks provide nutrients for plants and keep nutrients so as not to wash so it can be optimally used by plants. Nutrient availability is enough to make the plants are able to absorb the nutrients needed for growth. It is appropriate in the opinion of Leiwakabessy and Sutandi (2004) that the availability of nutrients has an important role on the growth and yield of rice, such as the availability of N, P and K. In addition, Chan et al. (2009) stated Award biochar chaff rice in paddy soil were carried out in order to increase the availability of nutrients indirectly also able to increase plant productivity.

## Conclusion

Based on the research that has been done can be concluded that application of rice husk biochar on paddysoil affecting the vertical distribution of nutrients and affect the physical, chemical and biological fields. This led to the dispersal patterns of nutrient uneven at each depth increases by 10 cm. Then,organic C at a depth of 0-10 increased from control to treatment F. The similar trend is also seen in the distribution of soil total N. In the layer below that spread differentials of each nutrient result of biochar application and type of tillage. Moreover, the content of P-available and Si-provided showed a pattern of escalating value of the top soil due to the effect of various doses of biochar chaff applied.

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## References

Adly, I. 2015.Effects of some wetland management system on soil physical properties. Essay. Faculty of Agriculture.Andalas University.

Bollich, P.K. and V.V. Matichenkov.Silicon 2002. Status of selected Louisiana rice and sugarcane soils. In Proceedings of the Second Silicon in Agriculture Coference, 22-26 August 2002, Tsuruoka, Yamagata, Japan. Pp 50-53.

Chan, Y. C., and Xu, Z. 2009. Biochar: nutrient properties. In: J. Lehmann and S. Joseph (eds), Biochar for environmental management. Publisher Earthscan.Pp 66-84.

Darmawan., Subagyo., Masunaga, T., and Wakatsuki, T. 2006. Effect of long-term intensive rice cultivation on the available silica content of rice soils; The case of Java Island, Indonesia. Soil Sci. Plant Nutr., 52, 745-753.

Febricio, A. R., and Lawrence E. D. 2005. Silicon and rice disease management. Fitopatol.bras., 30, 457-469.

Gani, Anischan. 2009. Potential Biological charcoal (biochar) as Material Shaping land. Food Crop Science, 4, 33-44.

Hanafi, K. A. 2013. Fundamentals of soil science. textbook writing incentive recipient of 2008 from the director and community services directorate general of higher education. King Grafindo Persada. Jakarta. 360 p.

Hardjowigeno, S., and Rayes, S. 2005. Soil rice (characteristics, conditions, and land issues rice in Indonesia). Bayumedia Publishing. Poor. 208 p.

Kimetu, J., Lehmann, H. J., Ngoze, S., Mugendi, D., Kinyangi, J., Riha, S., Verchot, L., Recha, J., and Pell, A. 2008. Reversibility of soil productivity decline with organic matter of differing quality degradation along a gradient. Ecosystems, in press. 442 pp.

Kishimoto, S., and Sugiura, G. 2009. Charcoal as a soil conditioner. Int Achieve Future 5 : 12-23 pp.

Kyuma, K. 2004. Paddy soil science. Kyoto University and Trans Pacific Press. Printed in Melbourne by BPA Print Group.380 pp.

Nurida, N. L., Dariah, A., and Rachman, A. 2010. The quality of agricultural waste as a raw material in the form of biocharpembenah for land rehabilitation. Proceedings of the National Seminar and Dialogue Land Resources. 2008. 209-215 p.

Safitri, L. 2015. Study of soil characteristics of rice on multiple systems land management in Pasaman

West Sumatra. Thesis. Andalas University's Graduate. 70 p.

# Embryogenic Callus Induction and Globular Embryo Formation of Kopyor Coconut (*Cocos nucifera* L.)

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## Abstract

Kopyor coconut is a mutant that can not be propagated by conventional technique because of the endosperm is damaged. Seedling propagation need a method to produce seedlings with the same character as its parent, uniform and can be produced in high quantities in a short time. Somatic embryogenesis technology has been used for mass propagation in plants. Plant regeneration through somatic embryogenesis needs some stages : embryogenic callus induction, callus proliferation, somatic embryo formation (globular, scutellar, and torpedo phase) and germination. This study aims to conducted the methods for embryogenic callus induction, callus proliferation, as well as the formation of somatic embryo structure at early stage (globular phase). Callus production was done using young zygotic embryo as the explants. Explant treatment was : intact embryo, halved longitudinally embryo, and thin slices embryo (0.1 cm). Media formulation used for callus induction were Eeuwens and Murashige Skoog (MS) with the addition of 2,4-D (10, 20, and 30 mg/l) and activated charcoal 2 g/l. Callus proliferation was done using Eeuwens and MS media containing picloram or 2,4-D (0.5, 2.5, 10 mg/l) and sucrose 30 g/l. The results showed that the use of halved embryo was more effectively used for callus production. Embryogenic callus conducted on Eeuwens medium added with 2,-4-D 30 mg/l and activated charcoal 2 g/l. Picloram showed less effectiveness in producing embryogenic callus compared to 2,4-D. Decreasing auxin concentrations into 0.5 mg/l combined with sucrose 60 g/l could induce embryogenic callus proliferation and formation of globular embryo.

Keywords: coconut kopyor, explant type, auxin, embryogenic callus induction, globular embryo

## 1. Introduction

Kopyor coconut is coconut endosperm mutants have lumpy loose from the shell. The endosperm is not normal which is very much favored by consumers because it is tasteful. Number of kopyor coconut plant is still limited, so selling price is quite expensive. Coconut kopyor can only be produced by a palm tree that has properties kopyor carried by a recessive gene pairs (kk). These properties do not appear if the recessive gene (k) paired with a dominant gene (k) [1] The absence of a palm tree kopyor true-to-type is caused by the inability of the embryo to germinate because of the damaged flesh as a backup source of food for the embryo. The only way to germinate the kopyor coconut is to grow in artificial media containing macro

nutrients, micro, vitamins, sucrose and a hormone in in vitro conditions.

Propagation of kopyor coconut can be performed using embryo culture techniques (embryo resque). By using this method can be obtained coconut kopyor which could produce 75-100% kopyor fruit per tree [1], however there are still many obstacles encountered in the process of culturing embryos coconut kopyor so that the percentage of success in obtaining seed is still relatively low at less than 30% [2]. Somatic embryogenesis is a technique of *in vitro* culture which can be used for mass production of seeds. Somatic embryogenesis provides an opportunity to produce crops from the somatic cells that can be produced by plants in large numbers.

Somatic embryogenesis (SE) is the process by which somatic cells, under induction conditions, generate embryogenic cells, which go through a series of morphological and biochemical changes [3], that result in the production of bipolar structure without vascular connection with the original tissue. The use of somatic embryogenesis method has been applied to a variety of crops such as cocoa [4], soybean [5] and coffee [6]. The use of somatic embryogenesis method for multiplication of the coconut kopyor seedlings have been reported by [7], but the percentage of embryos into plantlets formation remained low at 2.0 - 2.5%.

Several factors can affect the success of plant micropropagation through somatic embryogenesis are the type of explants, plant growth regulators, and the media formulation for embryogenic callus production, which has a high competency to regenerate into plants. Reference [8] stated that the difficulties in coconut regeneration are intense browning of tissues linked to Reviews their high sensitivity to synthetic auxins (eg 2,4-D), Considerable heterogeneity in tissue reaction low capacity for embryogenesis and caulogenesis ,

Somatic embryogenesis has a number of advantages over other techniques Because its synchronization of somatic embryo development can be Achieved, the resulting in a high level of clonal uniformity and genetic conformity, the which makes somatic embryogenesis is an essential method for the improvement of the majority of economically important plants ( [9], [10]). The concentration of sugar that could effect successes in somatic embryogenesis of plants because serves as the carbon source, it is also useful to maintain osmotic pressure of media. Regeneration via somatic embryogenesis consists of several stages : embryogenic callus formation, callus proliferation, maturation and germination of somatic embryo structure [11].The goal of the reasearch were to evaluate the callus embryogenic production of coconut kopyor and its development relative to the explants size, and the type and concentration of auxin.

## 2. Material and Methods

*2.1.Material*

The plant material used is young zygotic embryo of coconut kopyor.

2.2.*Metode*

*2.2.1. The effect of **e**xplant size and medium formulation on callus formation*

The immature embryos were sterilized with sodium hypochlorite (NaOCl) 1% and of 0.05% for 10 minutes and rinsed with sterile distilled water 3 times. The treatments tested were: 1) The size of explants : intact zygotic embryo, halved longitudinally excision of zygotic embryo, and cutting zygotic embryo to the size of 0.1 cm. 2) Media formulation for callus induction, were: a) Eeuwens + vitamin Morrel and Vettmore (MV) + 2,4-D (10, 20, 30 mg/l) + sucrose 30 g/l + activated charcoal 2 g/l, and b) Murashige -Skoog (MS) + 2,4-D (10, 20, 30 mg/l) + sucrose 30 g/l + activated charcoal 2 g/l. The culture were placed in a dark environment with a temperature of ± 25°. The design used was randomized complete design with 10 replications. The observation was done by

calculating the percentage of callus formation and callus growth. Callus growth was measured by performing scoring (1-4).

*2.2.2. Callus proliferation and maturation*

Callus proliferation was done using Eeuwens and MS medium containing 2,4-D or picloram (0.5, 2.5, and 10 mg/l), activated charcoal 2 g/l and sucrose 30 g/l. The culture was placed in a dark environment with a temperature of ± 25°C. Embryogenic callus formed were transferred to the medium to induce the formation of somatic embryo structure.

Media formulation used were 2,4-D 0.5 mg/l added with sucrose (30 and 60 g/l).The design used was randomized complete design with 10 replications. The observation was done by calculating the weight of callus, percentage of embryogenic callus formatuon, callus structure, callus colour and number of somatic embryo structures.

## 3. Result and Discussion

*3.1. The effect of **e**xplant size and medium formulation on callus formation*

Propagation of seedlings in large numbers is very important to get an economical propagation technology. Mass propagation of seedlings can be obtained by somatic embryogenesis technique. The initial step for the production of seeds through

Table 1. Callus formation and growth of coconut kopyor on different media formulations

| Explant size | Medium formulation (mg/l) | Callus formation (%) | Scoring of callus growth |
|---|---|---|---|
| Cutting embryo | Eeuwens + 2.4 D 10 +sukrosa 30 g/l | 40 | 1 |
| | Eeuwens + 2.4 D 20+sukrosa 30 g/l | 50 | 2 |
| | Eeuwens + 2.4 D 30 +sukrosa 30 g/l | 60 | 2 |
| | Murashige Skoog + 2,4-D 10+sukrosa 30 g/l | 50 | 2 |
| | Murashige Skoog + 2,4-D 20+sukrosa 30 g/l | 70 | 2 |
| | Murashige Skoog + 2,4-D 30+sukrosa 30 g/l | 90 | 2 |
| Halved excision embryo | Eeuwens + 2.4 D 10 +sukrosa 30 g/l | 80 | 2 |
| | Eeuwens + 2.4 D 20+sukrosa 30 g/l | 90 | 3 |
| | Eeuwens + 2.4 D 30 +sukrosa 30 g/l | 100 | 4 |
| | Murashige Skoog + 2,4-D 10+sukrosa 30 g/l | 70 | 3 |
| | Murashige Skoog + 2,4-D 20+sukrosa 30 g/l | 80 | 3 |
| | Murashige Skoog + 2,4-D 30+sukrosa 30 g/l | 90 | 3 |
| Intact embryo | Eeuwens + 2.4 D 10 +sukrosa 30 g/l | 100 | 2 |
| | Eeuwens + 2.4 D 20+sukrosa 30 g/l | 100 | 3 |
| | Eeuwens + 2.4 D 30 +sukrosa 3% | 100 | 4 |
| | Murashige Skoog + 2,4-D 10+sukrosa 30 g/l | 100 | 3 |
| | Murashige Skoog + 2,4-D 20+sukrosa 30 g/l | 100 | 3 |
| | Murashige Skoog + 2,4-D 30+sukrosa 30 g/l | 100 | 3 |

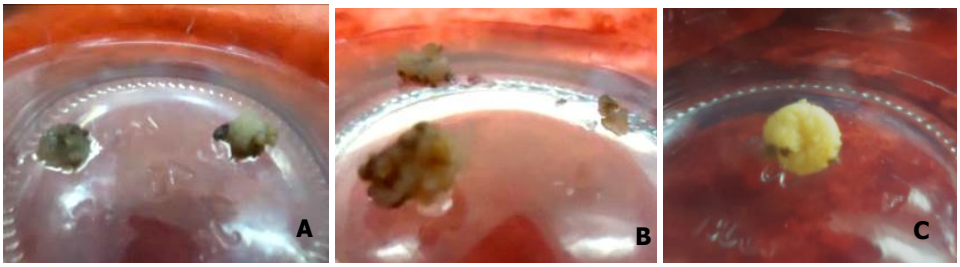


Fig.1. Callus formation of coconut kopyor with different sizes of explant A = 0.1 cm B = halved longitudinally excision
C = intact embryo

somatic embryogenesis is induced embryogenic callus.

The results showed that the callus could be formed on all media formulations used. Initiation of callus started to occur one months after planting. The use of 2,4-D 30 mg/l on MS or Eeuwens basic medium produced the highest callus formation (Table 1). Callus formation of halved excision and intact explant faster than cutting embryo (Fig. 1). Cutting embryo into a thin layer causing exudation of phenol compounds that inhibit the initiation and growth of callus. Reference [12] reported that callus browning occurs when explant explants released phenol compound into the media and oxidized. These compounds can inhibit enzyme activity and lead to browning media resulting in tissue death.

Reference [13] suggest that the induction of somatic embryogenesis in *A. andraeanum* cv. Eidibel is affected by the source of the explants and the type and concentration of the auxin. The most competent explants for the induction of embryogenic calli were derived from the petioles, but the whole leaf, leaf and root segments sectioned did not produced calli with embryogenic capacities, becoming oxidized after five weeks in culture.

The callus structure affect to plant regeneration success via somatic embryogenesis. Callus which were produced on all media formulation were compact and did not have embryogenic characteristic, therefore composition of growth regulators to obtain embryogenic callus must be conducted. According to [8] 2,4-D at the optimum concentration required to produce competent embryogenic callus. Endogenous hormone metabolism affected by genetic, physiological and environmental cues is well accepted in the induction phase of somatic embryogenesis [14]. Level of endogenous auxin is considered to be one crucial factors determining embryonic potensial of explant [15].

*3 2. Callus proliferation and maturation*

The highest callus weights resulting from treatment with the addition of 2,4-D 10 mg/l which is significantly different from other treatments (Table 2). The use of picloram did not gave a good results in callus proliferation of kopyor coconut compared with 2,4-D. Callus growth faster in medium added with 2,4-D (0.5, 2.5 and 10 mg/l) (Table 2).

The high efficiency of 2,4-dichlorophenoxy acetic acid (2,4-D) for induction of embryogenic response indicates a specific and unique character of this PGR. This synthetic growth regulator appear to act not only as an exogenous auxin analogue but also as an effective stressor [16].

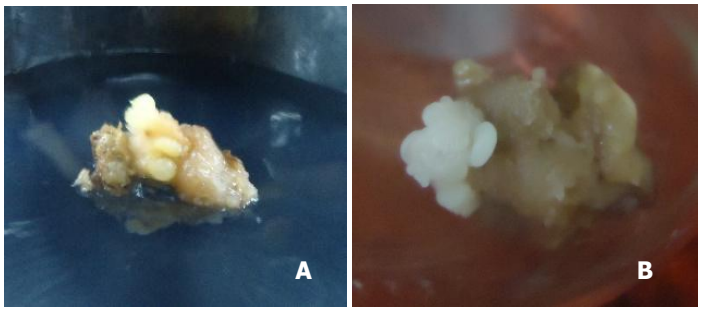


Fig. 2. Callus structure of kopyor coconut on Eeuwens medium containing 2,4-D 0.5 mg/l (A) and 2,4-D 10 mg/l (B)

Table 2. Callus proliferation of coconut kopyor in Eeuwens medium containing of 2,4-D with any concentration

| No | Media formulation (mg/l) | Callus weight (g) | Embryogenic callus (%) | Callus structure | Callus colour |
|---|---|---|---|---|---|
| 1. | Eeuwens (vitamin MV)+2,4D 0.5 mg/l + sukrosa 30 g/l | 0.83 b | 86.67 a | friable | Yellowish white |
| 2. | Eeuwens (vitamin MV)+2,4D 2.5 mg/l + sukrosa 30 g/l | 0.93 b | 73.33 b | friable | Yellowish white |
| 3. | Eeuwens (vitamin MV)+2,4D 10 mg/l + sukrosa 30 g/l | 1.30 a | 60.00 cd | compact | Yellowish white |
| 4. | Eeuwens (vitamin MV)+ pic 0.5 mg/l + sukrosa 30 g/l | 0.53 c | 66.67 bc | friable | Yellowish white |
| 5. | Eeuwens (vitamin MV)+ pic 2.5 mg/l + sukrosa 30 g/l | 0.70 bc | 53.33 d | friable | Yellowish white |
| 6. | Eeuwens (vitamin MV)+ pic 10 mg/l + sukrosa 30 g/l | 0.80 b | 53.33 d | compact | Yellowish white |

*Explanation : MV = Morrel and Vettmore*
*pic = picloram*

Table 3. Formation of globular structure on medium formulation containing 2,4-D with different sucrose concentration

| Media formulation | Callus weight (g) | Globular Structure | Colour |
|---|---|---|---|
| Eeuwens (vit MV)+2,4D 0.5 mg/l + sucrose 30 g/l | 1.13 a | 3 | Yellowish white |
| Eeuwens (vit MV)+2,4D 0.5 mg/l + sucrose 60 g/l | 1.26 a | 5 | Yellowish white |

*Explanation : MV = Morrel and Vettmore*

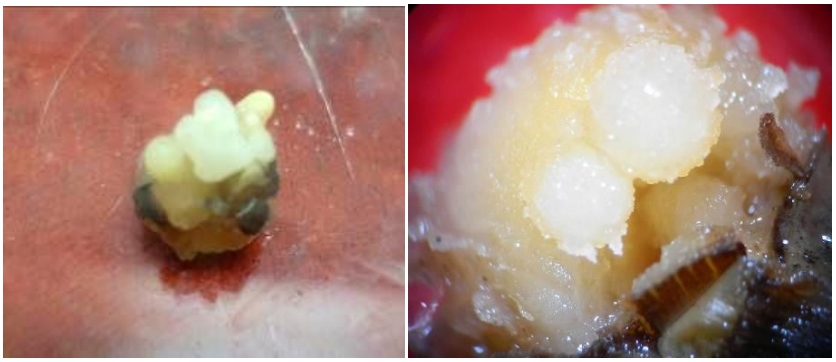

Fig. 3. Formation of globular structure in Eeuwens media containing 2,4-D 0.5 mg// and sucrose 60 g/l

The same results with [17] research which shows that the use of picloram less effective compared to 2.4 -D in coconut kopyor induce callus formation. Reference [18] reported that the use of 2.4-D and picloram with low concentrations (1.02 – 6.04 mg/l) has not been able to induce the formation of callus on zygotic embryo of kopyor coconut.

Decreasing auxin concentration could induce callus embryogenic formation, visible from the callus structure and color. The addition of 2,4-D or picloram (0.5 and 2.5 mg/l) produce friable callus with embryogenic character, while the use of 2,4-D or picloram 10 mg/l produce compact callus (Fig. 2). The highest embryogenic callus conducted on Eeuwens medium containing 2,4-D 0.5 mg/l which significantly different with the other treatments (Table 2).

Embryo structure maturation is strongly influenced by the concentration of auxin and sucrose. Decreasing concentration of 2,4-D to 0.5 mg/l could induced the initiation of somatic embryo formation. Callus formed yellowish-white and form a globular structure and glossy colored (Fig.3). The use of sucrose at a concentration of 60 g/l seems to be better in the formation of somatic embryo. The number of globular embryo was greater on medium containing sucrose 60 g/l than sucrose 30 g/l (Table 3). According to [8], higher sucrose concentration needed on somatic embryo formation.

## Conclusion

The use of halved longitudinally embryo was more effectively used for callus production. Embryogenic callus conducted on Eeuwens medium added with 2,-4-D 30 mg/l and charcoal 2 g/l. Picloram showed less effectiveness in producing embryogenic callus compared to 2,4-D. Decreasing auxin concentrations into 0.5 mg/l combined with sucrose 60 g/l could induce embryogenic callus proliferation and somatic embryo formation at early stage (globular phase).

## Acknowledgements

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## References

[20] Tahardi, J.S. 1997. Kelapa kopyor sebagai komoditi alternatif agribisnis. Warta Puslit Biotek Perkebunan. Bogor. Hal. 16-21.

[21] Mashud, N. 1999. Penerapan kultur jaringan pada tanaman kelapa. Makalah Simposium Hasil Penelitian Tanaman Kelapa dan Palma Lain. BALITKA. Menado.

[22] Quiroz-Figuera, F R, Rojas-Herrera, R.M. Galaz-Avaloz, and V.M. Loyola-Vargas. 2006. Embryo production through somatic embryogenesis can be used to study cell

differentiation in plants. Review paper. Plant Cell Tissue and Organ Culture. 86:285-301. DOI 10.1007/511240-006-9139-6.

[23] Quainoo, A.K., and I. B. Dwomon. 2012. The effect of absisic acid in the conversion of cacao somatic embryos into planlets. Frontiers in Science. 2(2): 6-10.

[24] Ranjitha Kumari, B.D.,A. Settu, and G. Sujatha.2006. Somatic embryogenesis and plant regeneration in soybean (Glycine max(L.) Merr). Indian.J.Biotechnol. %:243-245.

[25] Gatica,A.M., G. Arrieta, and Espinoza, A.M. 2008. Direct somatic embryogenesis in *coffea arabica* L. cvs. Caturra and Catuai : effect of triacontanol, light condition,and medium consistency. Agronomía Costarricense 32(1): 139-147.

[26] Sukendah. 2012. Induction and morphology of dwarf kopyor coconut (*Cocos nucifera* L.) embryogenic callus. Temu pakar bioteknology IPB 2012.

[8] Buffard-Morel, J., J.L. Verdeil, S. Dussert, C. Magnaval, C. Huet, and F. Grosdemane. 1995. Initiation of somatic embryogenesis in coconut (*Cocos nucifera* L.).

[9] Lu, J., J. Vahala, and N. A. Papine. 2011. Involvement of ethylene in somatic embryogenesis in Scots pine (*Pinus sylvestris* L.). Plant Cell, Tissue and Organ Culture, 107(1) : 25-33.

[10] Sivanesan, I., M. Lim, and B. Jeong. 2011. Somatic embryogenesis and plant regeneration from leaf and petiole explants of *Campanula punctata* Lam. var. Rubriflora Makino. Plant Cell, Tissue and Organ Culture. 107 (2) : 365-369

[11] Zimmerman, J.L. 1993. Somatic Embryogenesis : A Model for Early Development in Higher Plants. The Plant Cell 5 (10): 1411-1423.

[12] Laukkanen, H., H. Haggman, S. Kontunen-Soppela, and A. Hohtola. 1999. Tissue browning of *in vitro* cultures of Scots pine: Role of peroxidase and polyphenol oxidase. Physiol. Plant. 106:337-343.

[13] Kalimuthu, K., S. Paulsamy, R. Sentilkumar, and M. Sathya. 2007. In vitro propagation of the biodiesel plant *Jatropa curcas* L. Plant Tiss. Cult. Biotech. 17(2):137-147.

[14] Jimenez, V.M. 2005. Involvement of plant hormone and plant growth regolators in in vitro somatic embryogenesis. Plant Growth Regulation. 47:91-110.

[15] Feher, A., TP.Pasternak, and Dudits. 2003. Review of Plant Biotechnology and applied genetics. Transition of somatic plant cells to an embryogenic state. Plant Cell, Tissue, and Organ Culture. 74:201-228.

[16] Gaj, D., 2004. Factors influencing somatic embryogenesis induction, and plant regeneration with particular reference to *A. thaliana* (L.) Heynh. Plant Growth Regulation. 43:27-47.

[17] Sukendah. 2012. Induction and morphology of dwarf kopyor coconut (*Cocos nucifera* L.) embryogenic callus. Temu pakar bioteknology IPB 2012.

[18] Larekeng,S.H. N. AA. Mattjik, A. Purwito, dan Sudarsono. 2012. Penggunaan 2,4-D dan auksin untuk induksi kalus embriogenik tanaman kelapa kopyor secara in vitro. Temu pakar bioteknologi IPB 2012.

# The Role of Cow Manure to Reduce The Need of Nutrient N Inorganic In Banana Plant Vegetative Growth

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## Abstract

Banana is the one of important food in the world after rice, wheat, and maize. Most of agriculture land became poor in nutrient because the using in organic fertilizer. Organic agricultural was one of solution to increase soil organic matter, soil productivity and reduce cost production. The purpose of this research was to know the role of cow manure to reduce the needs of nutrient N inorganic in banana plant vegetative growth. This research was conducted at Aripan Experimental Field, Indonesian Tropical Fruit Research Institute, West Sumatra, Indonesia at an altitude of ± 415 m above the sea level. This research began in June 2015 until December 2015. The materials used in this research were Ketan variety banana sedlings, urea, SP36, KCl, and manure. The research design was Randomize Complete Block Design with 5 treatments and 3 replications, so all experiments unit consisted of 15 plots (each plot consisted of 5 sample plants). The treatment was the combination of manure with N inorganic fertilizer with multiple doses: 1) 100% N inorganic, 2) 75% N inorganic, 3) 50% N inorganic, 4) 25% N inorganic and 5) 0% N inorganic. The result showed that application cow manure 12,5 ton/ha was already substitute N inorganic until 75% for banana plant vegetative growth (plant height, stem circumference, shoot number, shoot length and shoot width).

Keywords : banana, cow manure, nutrient N, and vegetative growth

## 1. Introduction

Banana is the one of important food in the world after rice, wheat, and maize. This commodity has a good prospect for consuming where they are produced and for exported. Generally, banana production in Indonesia still in low condition because of less maintenance, less technology, and low input. Intensive treatments of banana plant with good maintenance, high input, and technology can increase banana production.

The growth and development of plant depend on soil properties and the input which given to the soil. Soil with low nutrient will make plant growth slowly and can't reach maximum growth rate. This condition need more input nutrient from outside to makes plant growth normally. One solution for this condition is using fertilizer to support plant to get nutrient.

Fertilization has significant impact to the agriculture activity. The farmer usually used fertilizer to increase plant growth and plant productivity, and also increase benefit from the agriculture activity. The farmer usually used inorganic fertilizer because it was easy to use and absorbed quickly by the plant [1]. But, application of inorganic fertilizer in a long term makes a side effect for soil. The side effect makes soil will not get in a good health, physics and biological soil will broken, and causing environmental pollution [2].

**Table 1.** Addition of plant height, stem circumference, shoot number, shoot length and shoot width of banana in combination of cow manure and N inorganic dosage treatment 5 MAA (Month After Aplication)

| Treatment | Addition of Plant Height (cm) | Addition of Stem Circumference (cm) | Addition of Shoot Number (pcs) | Addition of Shoot Length (cm) | Addition of Shoot Width (cm) |
|---|---|---|---|---|---|
| 100% N inorganic | 99,5 a | 24,1 a | 17,4 a | 109,1 a | 35,6 a |
| 75% N inorganic | 81,8 ab | 19,7 a | 16,7 a | 110,7 a | 31,2 a |
| 50% N inorganic | 80,6 ab | 22,6 a | 17,3 a | 99,6 a | 34,0 a |
| 25% N inorganic | 84,7 ab | 21,6 a | 17,1 a | 100,7 a | 31,2 a |
| 0% N inorganic | 75,7 b | 20,0 a | 17,3 a | 94,7 a | 31,9 a |

The other problem was the increase of inorganic fertilizer price. The farmer tries to find another alternative to increasing agriculture productivity without adverse impact for soil and environment. Organic agricultural was one of solution for the problem. Organic fertilizer can increase soil organic matter, soil productivity and reduce cost production [3,4,5]. The other benefit using organic fertilizer was the product contains more vitamin C, iron, phosphorus and fewer nitrates [6].

It has been widely reported the successful research result using organic fertilizer. It occurs in Amaranths [2], lettuce [1], sugarcane [7], maize [8], garlic [9], and tomato [10]. The purpose of this research was to know the role of cow manure to reduce the needs of nutrient N inorganic in banana plant vegetative growth.

## 2. Material and Methods

This research was conducted at Aripan Experimental Field, Indonesian Tropical Fruit Research Institute, West Sumatra, Indonesia at an altitude of ± 415 m above the sea level. This research began in June 2015 until December 2015. The materials used in this research were Ketan variety banana sedlings, urea, SP36, KCl, and manure.

The research design was Randomize Complete Block Design with 5 treatments and 3 replications, so all experiments unit consisted of 15 plots (each plot consisted of 5 sample plants). The treatment was combination of manure (12,5 tons/ha) combine with N inorganic fertilizer with multiple doses: 1) 100% N inorganic, 2) 75% N inorganic, 3) 50% N inorganic, 4) 25% N inorganic and 5) 0% N inorganic (100% N inorganic = 250 g N/plant).

The implementation of this research was planting, labeling, fertilization (based on treatment), and maintenance (weeding, watering, pest and disease control). Observations were: plant height, stem circumference, shoot number, the length of shoot and width of shoot. Observations were made every month after application. Data were analyzed statistically by F test and if the results F count larger than F table value of 5%, followed by HSD at the 5% significance level.

## 3. Result and Discussion

The treatment combination in this research gave different effect for the banana vegetative plant growth. Plant height, stem circumference, shoot number, the length of shoot and width of shoot banana plant generally increased in 5 MAA (Month After Application). The treatment combination of cow manure and 100% N inorganic showed the best result for an addition of plant height, an addition of plant girth, an addition of shoot number and addition shoot width (Table 1).

In 5 MAA, cow manure treatment without N inorganic was significantly lower in addition of plant height than other treatment. The best result for an addition of

banana plant height was cow manure treatment with 100% N inorganic but has nothing different effect than 75% N inorganic, 50% N organic and 25% N inorganic treatment. An addition of plant height range about 75,7 cm until 99,5 cm.

All treatment combination cow manure with N inorganic showed slow growth in the early month after application for all type of parameters (plant height, stem circumference, shoot number, shoot length and shoot width) and after that increased growth normally (Figure 1). The slow growth of the banana plant in first month after application connected with the initial physiological change associated with transplanting, it also reported in Amaranths plant [2].

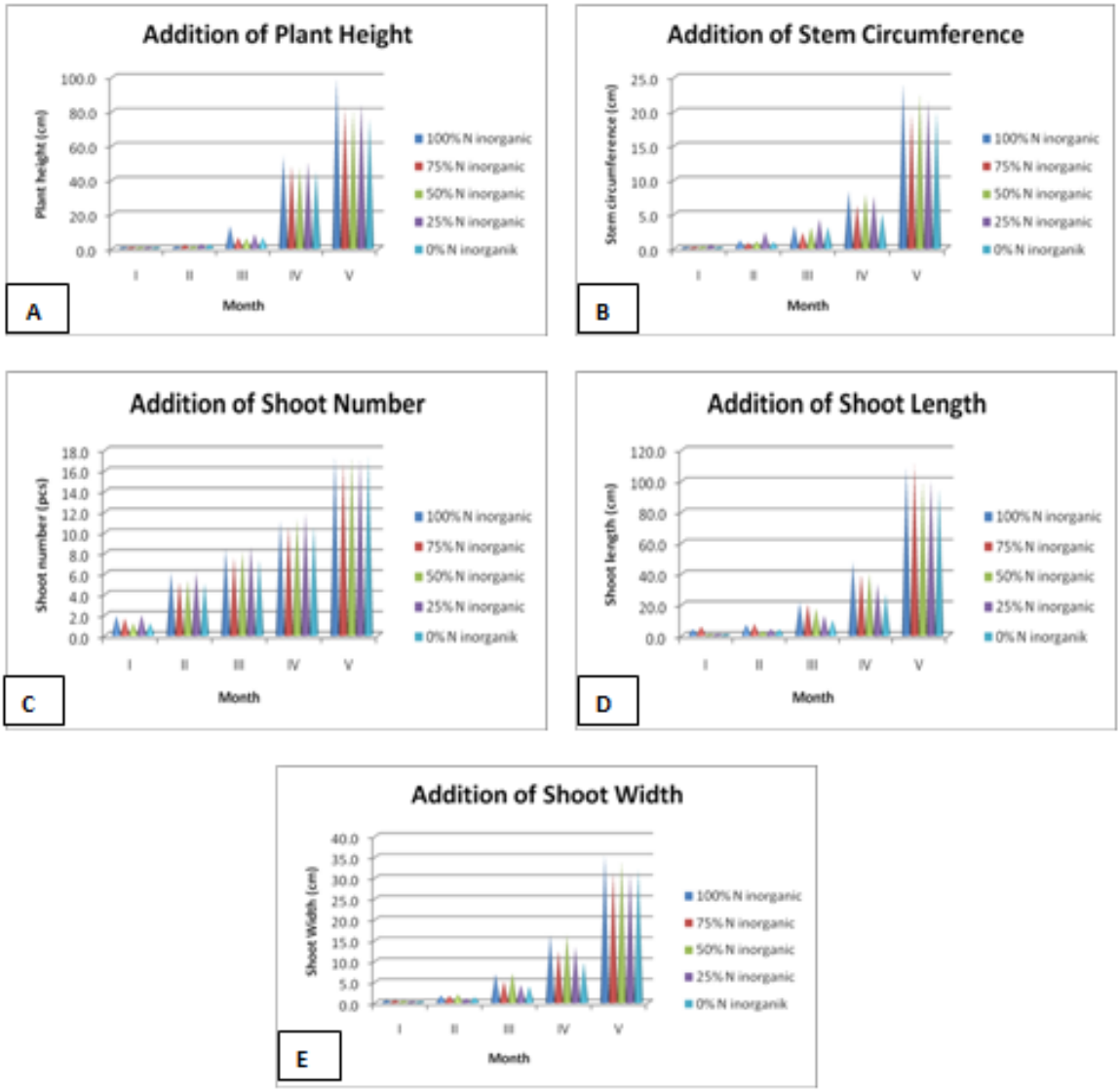


**Figure 1.** **A.** Addition of banana plant height 1 month after application until 5 month until application, **B.** Addition of stem circumference 1 month after application until 5 month until application, **C.** Addition of banana shoot number 1 month after application until 5 month until application, **D.** Addition of banana shoot length 1 month after application until 5 month until application, **E.** Addition of banana shoot width 1 month after application until 5 month until application.

The banana plant height growth slowly in first and second month (Figure 1), but after that increase significantly. Treatment combination of cow manure with 100% N inorganic dominance in addition of plant height start from 3 month after application until last research (5 month after application), but the increase of plant height is followed by three other treatments (75% N inorganic, 50% N inorganic and 25% N inorganic).

The growth of banana stem circumference also slow in first and second month after aplication, but also increase in three month after aplication. This phenomenom also happened to shoot length and shoot width. The addition of shoot number was increase in all treatments in two months after application. In the end of experiments, the other four parameters (stem circumference, shoot number, shoot length and shoot width) of banana plant has no different in growth rate.

The application of organic fertilizing, such us cow manure, poultry manure, pig manure, compost, sheep manure, etc gave different effect for plant. In lettuce, using chicken manure more suitable and productive than using inorganic fertilizer [1]. Fertilizing sugarcane with cow manure gives positive effect for plant, keep chemical composition and improve soil fertility [7]. Experiment in Amaranths showed that using organic or inorganic fertilizer can improve soil fertility, but inorganic fertilizer gave better performance in growth than using organic fertilizer [2].

The treatments combination cow manure with 25% N inorganic have the same effect with the combination treatments cow manure with 100% N inorganic in 5 type parameter vegetative growth of banana plant. So, application cow manure 12,5 ton/ha already substitute N inorganic until 75% for banana plant vegetative growth. In this research showed that cow manure can't fulfill the needs of all N nutrient, it must combine with 25% N inorganic.

Using cow manure or another organic manure for agriculture activity has a good impact to plant, soil and an environment. It can be used for nutrient source on plant, improve soil quality in one area and also have an important role in soil biology, chemistry and physics [9,10,11,12]. N in cow manure compost release slowly and kept over in a longer time, so it will be use for sustainable agriculture [13].

## Conclusion

Application cow manure 12,5 ton/ha already substitute N inorganic until 75% for banana plant vegetative growth.

## References

[1] M.T. Masarirambi, P. Diamini, P.K. Wahome and T.O. Oseni, "Effect of Chicken Manure on Growth, Yield and Quality of Lettuce (*Lactuca sativa* L.) 'Taina' Under a Lath House in a Semi Arid Sub Tropical Environtment". American-Eurasian J.Agric & Environ.Sci., vol. 12(3), pp 399-406, 2012.

[2] S. Oyedeji, D. A. Animasaun, A.A. Bello, and O.O. Agboola, "Effect of NPK and Poultry Manure on Growth, Yield and Proximate Composition of Three Amaranths". Journal of Botany., vol 2014, 6 pages, 2014.

[3] M. Mohanty, N.K. Sinha, K.S. Reddy, R.S. Chaudhary, A.S. Rao, R.C. Dalal, and N.W. Menzeis, "How Important is the Quality of Organic Amandments in Relation to Mineral N Avaibility in Soils?." Agric Res., vol. 2(2), pp 99-110, 2013.

[4] I.N.Y. Supartha, G. Wijana dan G.M. Adnyana, "Aplikasi Jenis Pupuk Organik pada Tanaman Padi Sistem Pertanian Organik". E-Jurnal Agroekoteknologi Tropika., vol. 1(2), pp 98-106, 2012.

[5] A.B. Almeida Júnior, C.W.A. Nascimento, M.F. Sobral, F.B.V Silva, and W.A. Gomes, "Soil Fertility and Uptake of Nutrients by Sugarcane Fertilized with Filter Cake". Rev. Bras. Eng. Agríc. Ambient., vol. 15(10), pp 1004-1013, 2011.

[6] V. Worthington, "Nutritional Quality of Organic Versus Conventional Fruits, Vegetables, and Grains". The Journal of Alternative and Complementary Medicine., vol. 7(2), pp 161-173, 2004.

[7] G. Guimaraes, R. de Paula Lana, R.C. Rodrigues, C.M. Veloso, R. de Souza Reis, M.R. de Miranda

Souza, and S. de Almeida Campos, "Growth and Composition of Sugarcane and Chemical Attributes of the Soil by Fertilizing with Different Level of Cow Manure". African Journal of Agriculture. Vol. 11(40), pp 3984-3990, 2016.

[8] D.T. Guerena, J. Kimetu, S. Riha, H. Neufeldt, and J. Lehmann, "Maize Productivity Dynamics in Response to Mineral Nutrient Additions and Legacy Organic Soil Inputs of Contrasting Quality". Field Crop Res., vol. 188, pp 113-120, 2016.

[9] S. Suthar, "Impact of Vermicompost and Composted Farmyard Manure on Growth and Yield of Garlic (*Allium sativum* L.) Field Crop". International Journal on Plant Production., vol. 3(1), pp 27-38, 2009.

[10] M. Mehdizadeh, E.B. Darbandi, H. Naseri-Rad, and A. Tobeh, "Growth and Yield of Tomato (*Lycopersicum esculentum* Mill.) as Influenced by Different Organic Fertilizer". International Journal of Agronomy and Plant Production., vol. 4(4), pp 734-738, 2013.

[11] Q. Liang, H. Chen, Y. Gong, M. Fan, H. Yang, R. Lal, and Y. Kuzyakov, "Effect of 15 Years of Manure and Inorganic Fertilizer on Soil Organic Carbon Fraction in a Wheat-Maize System in the North China Plain". Nutr Cycl Agroecosyst., vol. 92, pp 21-33, 2012.

[12] S.R. Wilkinson, "Plant Nutrient and Economic Value of Animal Manures". Journal of Animal Science., vol. 48(1), pp 121-133, 1979.

[13] L. Chen, W.A. Dick, J.G. Streeter, H.A.J. Hoitink, "Ryegrass Utilization of Nutrient Release from Composted Biosolids and Cow Manure". Compost Science and Utilization., vol. 4(1), pp 73-83, 1996.

# Study The Physical And Chemical Properties Of Bioethanol From Pineapple Skin *(Ananas comusus* L.Merr *)*

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## Abstract

This research aims to study the physical and chemical properties of bioethanol from pineapple skin ( *Ananas comusus* L. Merr ).This study uses a completely randomized design (CRD), which consists of two factors: the first factor the amount of yeast ( $R_1$ = 0.1 % , $R_2$ = 0.2 %, $R_3$= 0.3 % , $R_4$ = 0.4 % ) and factor the second fermentation time ( $L_1$ = 1 day , $L_2$ = 2 days , 3 days = $L_3$ , $L_4$ = 4 days ) . The parameters analyzed were total soluble solid, alcohol content, with a yield, total acid, and seconds flame. The result showed that the amount of yeast had highly significant effect on the total soluble solid, alcohol content, with a yield, total acid, and second flame. Fermentation time had highly significant effect on total soluble solid, alcohol content, with a yield, total acid, and second flame. The interaction of amount of yeast and fermentation time had a highly significant effect on total soluble solid and alcool content.

*Keywords :Pineapple Skin ,Saccharomyces cerevisiae , Bioethanol*

## 1. Introduction

Estimates of the decline of petroleum products in the future and a greater dependence to petroleum energy sources, encouraging research and development of alternative sources of energy from the source of the updated. Solid waste utilization of palm oil plantations like oil palm shells as raw material in the manufacture of briquettes have contributed in the procurement of renewable alternative energies, [3]. Pineapple skin much waste generated from pineapple fruit processing industry.Pineapple fruit processing industry can be the result of waste up to 135 thousand tons of the year [3]. General waste pineapple untapped optimally and just dumped it, especially the part of the skin, as this section pertained could not be consumed but the potential benefits in the manufacture of bioethanol.

Bioethanol is alternative energy sources have a good prospect as remote liquid fuel with renewable raw materials, eco-friendly and economically very beneficial macro against rural communities because it can be produced from waste pineapple skin easily obtained and not to take advantage. [3], Stated that the long fermentation of the most optimal for manufacturing process bioethanol is 3 days. If the fermentation is done over 3 days, thus the levels of alcohol can be reduced. The reduced levels of alcohol because alcohol has been converted intoother compounds, such as esters.

Fermentation is defined as a process of oxidation, reduction in biological systems produce energy which as electron donors and aseptor use of organic compounds. Organic compounds are converted into a series of reactions that are catalysed by enzymes into a form to another, such as pillars, alcohol and if there were further oxidation forms acids [1].

One type of microorganism that has power conversion of sugars to ethanol is very high is Saccharomycesscereviceae. Utilizing Saccharomyces cereviseae Human to make a

fermented, good food and drinks containing alcohol. Types of microbes is capable of changing a liquidcontaining sugar into alcohol and CO2 gas quickly and efficiently [3].To obtain high-quality bioethanol b one of them done by distillation.

The principle of distillation process which separating ethanol from a mixture of ethanol and water. For a solution that consists of different components of the real temperature of the boiling, distillation is the easiest way to operate and also a way of separation that are thermal efficient. At atmospheric pressure, water boils at a temperature of 100 °C and ethanol boils at a temperature of about 77 °c. The difference in the boiling point that allows the separation of a mixture of ethanol-water [1].

## 2. Materialand Methods

*2.1. Research Place*

Research was conducted in the laboratory of Agricultural Technology of the Faculty of Agriculture Field UMSU. Ingredients: pineapple rind of marketer pineapples in Medan. Microbes fermentation: Saccharomyces cerevisiae instant yeast obtained from the traditional market in Medan.Chemicals: NaOH 0.1 N, Aquades, Indicator PP 0.1%. Tools: knife, eyedropper, becker glass, blender, pots, cloth filter, stove, bottles, mortalpiknometer, biuret, erlenmeyer flask, pipette drops, hand-refraktometer, distillation.

*2.2. Creation of StaterSaccharomyces cerevisiae*

Prepared aquades as much as 1000 ml into a beaker glass, then add granulated sugar 100 g (10%), homogeneous concentration using magnet stirer, then carried out sterilization by autoclave at 121$^{o}$C for 30minutes then allow to cool, then add the yeast 5% then incubation at room temperature 28-30$^{o}$C for 18 hours [1].

*2.3. Creation of Bioetanol*

Shredded pineapple skin and then blended, then added little by little water, pureed pineapple skin filtered up to gained 300 g pineapple skin cider with a 1:1 comparison, add 51 g sugar (17%), the solution is boiled to boiling and then chill, stater added appropriate treatment into an erlenmeyer flask, then added pineapple juice into an erlenmeyer flask, erlenmeyer flask then closes with bottle cap that has been perforated and hose (hose should not touch the pineapple juice) erlenmeyer flask, then closed with duct tape so that no air is coming in, and then do the fermentation of appropriate treatment. This study used a Randomized Complete Design (RAL), which is comprised of two factors, namely the first factor is the amount of yeast and the second factor long fermentation.

Table 1. The influence of the number of observed Parameters against Yeast

| Parameters Of Observation | Influence of amount of yeast (R) | | | |
|---|---|---|---|---|
| | $R_1$ (0,1%) | $R_2$ (0,2%) | $R_3$ (0,3%) | $R_4$ (0,4%) |
| Total dissolved solids ($^{o}$Brix) | 11,15 $^{a,A}$ | 9,91 $^{b,B}$ | 9,19 $^{c,C}$ | 7,71 $^{d,D}$ |
| Alcohol levels (%) | 36,5125 $^{b,B}$ | 29,8825$^{c,C}$ | 42,2125$^{a,A}$ | 42,2525$^{a,A}$ |
| Yield (%) | 0,9575 $^{c,C}$ | 1,0338 $^{bc,BC}$ | 1,2100 $^{b,AB}$ | 1,4600 $^{a,A}$ |
| Total acids (%) | 0,8000 $^{b,C}$ | 1,2100 $^{a,B}$ | 1,2800 $^{a,A}$ | 1,5200 $^{a,A}$ |
| Flame time (seconds) | 2,83 $^{d,D}$ | 4,42 $^{c,C}$ | 6,41 $^{a,A}$ | 6,03 $^{b,B}$ |

Description: a different letter Notation on the same line shows the influence of different very real at the 5% level (lowercase) and different very real at the 1% level (uppercase)

Table 2. Long Fermentation influence against the observed Parameters

| Parameters Of Observation | The influence of long fermentation (L) | | | |
|---|---|---|---|---|
| | $L_1$ (1 hari) | $L_2$ (2 hari) | $L_3$ (3 hari) | $L_4$ (4 hari) |
| Total dissolved solids ($^o$Brix) | $10,13^{a,A}$ | $9,55^{b,B}$ | $9,31^{c,C}$ | $8,98^{d,D}$ |
| Alcohol levels (%) | $26,5438^{d,D}$ | $33,1800^{c,C}$ | $40,5275^{b,B}$ | $50,6088^{a,A}$ |
| Yield (%) | $0,9625^{c,C}$ | $1,0900^{bc,BC}$ | $1,2225^{ab,AB}$ | $1,3863^{a,A}$ |
| Total acids (%) | $0,9600^{b,B}$ | $1,2000^{a,A}$ | $1,1200^{a,A}$ | $1,4400^{a,A}$ |
| Flame time (second) | $3,70^{b,B}$ | $4,47^{b,B}$ | $5,44^{a,A}$ | $6,09^{a,A}$ |

Description: a different letter Notation on the same line shows the influence of different very real at the 5% level (lowercase) and different very real at the 1% level (uppercase)

If the obtained results differ markedly from the very real and these two factors then the test is continued with a different test, using the test. Least Significant Range (LSR CARS).

## 3. Result and Discussion

### *3.1. The influence of the number of observed Parameters against Yeast*

On the basis of the research that has been done, the amount of yeast gives the effect on total dissolved solids, alcohol levels, yield, total acids, flame time as shown in table 1.

### *3.2. Long Fermentation influence against the observed Parameters*

On the basis of the research that has been done, long fermentation gives influence to the total dissolved solids, alcohol levels, yield, total acids, flame time as could be seen on Table 2.

### *3.3. Total dissolved solids ($^o$Brix)*

In table 1 it can be seen the amount of yeast give different very real influence ($p < 0.01$) to the total dissolved solids. According to [3], yeast Saccharomyces cerevisiae producing enzymes zimase. Enzymezimase serves to overhaul the sucrose into Monosaccharides (glucose and fructose). The higher the concentration of yeast, then the morethe amount of yeast that is found in fermented materials.

In table 2 it can be seen long fermentation give different very real influence ($p<0,01$)to the total dissolved solids. According to [1], the fermentation is an shake-up of materials containing carbohydrates intoMonosaccharides, alcohol, acetic acid, carbon dioxide and other compounds. On the process of alcoholic fermentation, glucose completely revamped into alcohol, carbon dioxide and $H_2O$. This shows that, the longer the fermentation process lasts, the more glucose is converted into other compounds, so that TSS generated increasingly declining.

The interaction of the amount of yeast and long fermentation give different very real influence ($p<0,01$) to the total dissolved solids. According to [1], the Yeast Saccharomyces cerevisiae producing enzymes zimase. Enzyme zimase serves to overhaul the sucrose into Monosaccharides (glucose and fructose). The amount of Yeast fermentation with the old 0.1% 1 day yeast has a population of at least compared to the other, so that glucose does not completely overhauled into alcohol, whereas the amount of Yeast fermentation with the old 0.4% 4 days, producing the lowest remaining TSS, so that shows that the higher the concentration of yeast, glucose reshuffle more and more.

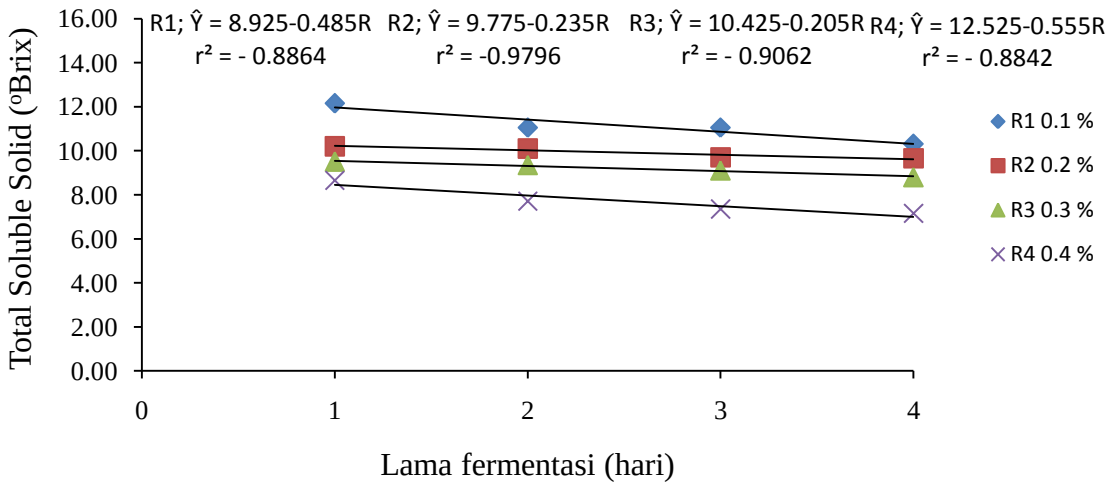


Figure 1. The relationship of interaction amount of yeast and long fermentation to the total dissolved solids (°Brix)

*3.4. Alcohol Levels (%)*

In table 1 it can be seen the amount of yeast give different very real influence ($p < 0.01$) against the levels of alcohol. The greater the concentration of yeast is given then the greater the amount of yeast in the fermentation of Saccharomyces cerevisiae primarily materials. Yeasts which remodel the simple candy found in the juice into alcohol through the process of fermentation. With the amount of Yeast fermentation in the 0.2% decline rate of alcohol which is formed, this is due to the amount of Yeast fermentation in 0.1% allegedly are toxic, so the yeast cell growth is compromised, the dissolved oxygen decreased and cell growth was too dense, so that the substrate is not sufficient for growth, as a result of the formation of alcohol also decreases.

In table 2 it can be seen long fermentation give different very real influence ($p<0.01$) against the levels of alcohol. According to [1] fermentation is a change up by enzymes that are produced by yeasts, that include change of starch into sugar, and sugar into alcohol and carbon dioxide. One of the kinds of microorganisms which have a very high sugar conversion is Saccharomyces cerevisiae, fermentation can be interpreted as a change in way up by the enzyme some bacteria, yeasts and fungi. Examples of chemical changes from fermented milk acidification, decomposition of starch and sugars into alcohol and carbon dioxide, as well as the oxidation of organic nitrogen compounds. In this case, the longer the fermentation process takes place, then the number of carbs overhauled into simpler compounds (glucose) more and more. According to [1], the longer the fermentation the more glucose that overhauled into alcohol, so that the resulting alcohol levels higher.

The interaction of the amount of yeast and long fermentation give different very real influence ($p < 0.01$) against the levels of alcohol. According to [3], the longer the fermentation process and the more doses of yeast given bioetanol-increasing levels then.The highest levels of ethanol fermentation 3 days at a time due to the activity of Saccaromycescerevisiaekhamir that works optimally as well as enzimatis which is not obstructed. The fermentation time influence on result because the longer the fermentation will increase levels of bioetanol, however if too long fermentation nutrients in the substrate will be exhausted and Saccaromycescerevisiae yeasts can fer-

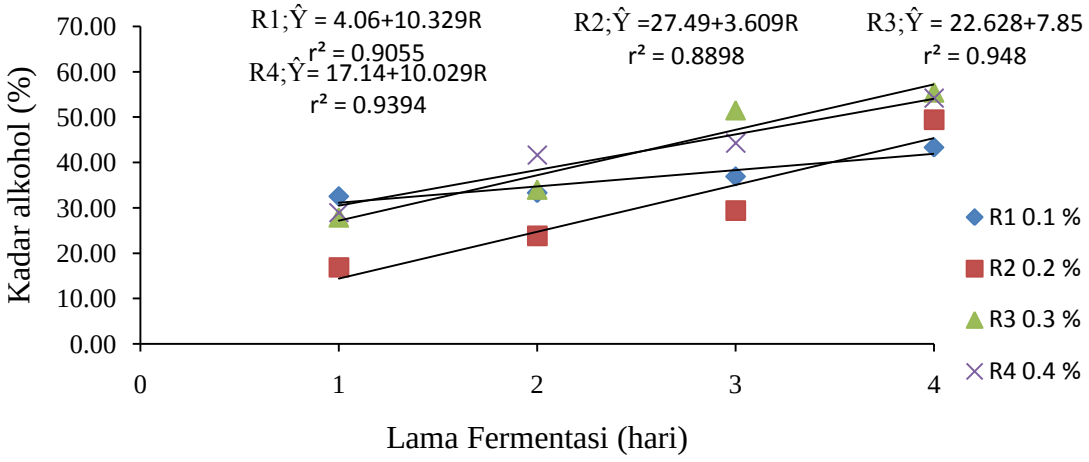


Figure 2. The relationship of interaction amount of yeast and fermented old levels alcohol

ment materials no longer so Saccharomyces cereviceae kekurangan makanan cereviceae Saccharomyces performance resulting in decreased and resulted in levels of bioetanol produced will decrease also [3].

*3.5. Yield (%)*

In table 1 it can be seen the amount of yeast give different very real influence ($p<0.01$) against yield. This is the case the higher amount of yeast is added, then the more yeast grows and breeds and will accelerate an overhaul of glucose into alcohol. According to [1], the more the amount of glucose present in the material, the higher the amount of alcohol produced from glucose reshuffle. The greater the amount of starch which is hydrolysed into glucose, and more and more microbes change glucose into alcohol, as a result of the resulting alcohol levels higher.

In table 2 it can be seen long fermentation give different very real influence ($p < 0.01$) against yield. The longer fermentation then the greater levels of alcohol, so that the greater the amount of ethanol produced anyway and vice versa. This happens because the fermentation process that generally occurs during the 2-4 days. This statement is in accordance with the literature from [3] stating that the fermentation takes about 28-72 hours.

*3.6. Total Acids (%)*

In table 1 it can be seen the amount of yeast give different very real influence ($p < 0.01$) to the total acid. [3] States that organisms that produce the acid lactic acid as well as other alcohol or acid are known as heterolaktat or hetero fermentation and often using the phosphate pentosa so that the greater percentage of the yeast then the number of acid. The higher the number, the more yeast yeastcontained in the fermentation medium, so the more carbs overhauled into alcohol and other compounds.

In table 2 it can be seen long fermentation give different very real influence ($p < 0.01$) against yield. [14] suggests that in this phase many microbes grow and divide so that the numbers are rising quickly. The longer the fermentation of Saccharomyces cerevisiae then will be more active to change the alcohol into acetic acid acid amount will be higher, in the manufacture of the alcoholic fermentation process involving alcohol and acetic acid fermentation continuously. Acetic acid is essentially an advanced fermentation product of alcoholic fermentation. Before the creation of alcohol raw material used looks a

little more viscous but after experiencing the process of fermentation to alcohols and acids, the shape is being diluted and it smelled very sour.

*3.7. Flame time (seconds)*

In table 1 it can be seen the amount of yeast give different very real influence ($p < 0.01$) with respect to time of fire. Decline at level R3 it is caused that the content of the material contained on the bioetanolis experiencing a downturn, this is due to bacteria Saccharomyces cerevisiae can't overhaul the sugar into alcohol, so that the overall activity of bacteria to convert glucose into decline.

In table 2 it can be seen long fermentation give different very real influence ($p<0.01$) with respect to time of fire. From 4 variations of long fermentation of pineapple skin, note that the length of time the fermentation of Saccharomyces cereviseae cell number affects the depression. The longer fermentation, then the more the amount of ethanol that is formed, and vice versa. It is marked with a volume of destilat containing ethanol and simply applied the increase over time of fermentation.

## Conclusion

1. The amount of yeast and long fermentation give different very real influence on the extent of p to the total 0.01 < dissolved solids (TSS), total acids, alcohol, yield levels, and the time of fire.
2. Interaction of the amount of yeast and long fermentation give different influences very real to the total dissolved solids (TSS) and the levels of alcohol

## Refernces


[1]. Ardilla, D, Bahrin, M.Taufik., 2011. Study Additive Addition To The Calorific Value Briquettes Shells Palm Oil. Agrium Journal. Page 116-123

[2] Asngad, Aminah and Suparti. (2011). "Long Fermentation and Yeast Different Doses on Fermentation Gaplek Cassava (*Manihotutilissima, Pohl*) Mukibat Varieties Of Glucose and Bioethanol".

**[3]**. Desrosier, 1988. Food Preservation Technology.Translator M. Muljohardjo.UI-Press, Jakarta.

[4] Hidayat, N., M. and S. C. PadagaSuhartini, 2006. Industrial Microbiology.Andi, Yogyakarta.

[5] Iida, T., H. Izumida, Y. Akagi, and M. Sakamoto. (1993), "Continuous ethanol fermentation using z in molasses medium.mobilis immobilized in photo-crosslinkable resin gels ", Journal of fermentation and bioengineering, Vol. 75, No. 1, 32-35.

[6] Judoamidjojo, M., A.A. Dervish, and E.G. Said. 1992. Fermentation Technology. Jakarta: Rajawali Press.

[7] Lurgi, G., 1989. Alcohol Destilation, Gulf Publishing Company, Frankur

[8] Nurhayati. 2013. The appearance of broilers that consumed feed containing flour supplemented with yogurt pineapple skin. Agripet.Vol 13 2: 15-20

[9] Sari, I. M., Noverita and Yulneriwarni. 2008. Utilization of rice straw and grass --- grass in ethanol fermentation using fungi and yeasts *Trichodermaviridae, Saccharomycesscerevisiae*. Vis Vitalis.5 (2): 55 -- 62.

[10]. Setyohadi, 2006. Food Microbiology Process (Process Damage and Processing).USU-Press, Medan.

[11] Setyawati, H., R.A.Nanik. 2013. Bioethanol From Skin Pineapple With Variations Massa *Saccharomycescerevisiae* and the Fermentation Time. Department of Chemical Engineering, Faculty of Industrial Technology, National Institute of Technology, Malang.

[12] Syarief,2009. Different of Vinegar and Khamir.http: //halalsehat.com//journal/item. Accessed on March 4, 2016.

[13] Torrora, G. J., R. Funke, B. and L. Case C., 2001.Fundamental of Microbiology.Addison Wesley Longman, Inc., New York.

[14] Tipteerasri, T., W. Hanmoungjai dan P. Hanmoungjai. 2009. Ethanol Production from Crude Whey by *Kluyveromyces marxianus* TISTR5695.Chiang MaiUniversity, Thailand.

[15] Winarno.F.G., S. and D. FardiazFardiaz, 1992. Nutrition Food Chemistry.Gramedia Pustaka Utama. Jakarta.

[16] Winarno, F.G. and S. Fardiaz. 1990. Biofermentation and protein biosynthesis. Publisher: Space. Bandung.

# Increase Moringa Leaf Powder and Long Roasting on Protein Content in the Making of Cookies from Mocaf *(Modified Cassava Flour)*

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## Abstract

This study aims to determine the effect of addition Moringa leaf powder and long roasting on protein content in the making of cookies from mocaf. The research uses Complete Randomized Design (CRD) factorial with (2) two replication. Factor I is addition of moringa leaf powder with password (T) which consists of 4 standard, namely : $T_0$ = 0%, $T_1$ = 3%, $T_2$ = 6%, and $T_3$ =9%. Factor II is long roasting with password (L) which consists of 4 standard, namely : $L_1$ = 5 minute, $L_2$ = 10 minute, $L_3$ = 15 minute, and $L_4$ = 20 minute.The results of statistical analysis gave the following conclusion: the highest protein 7.096% contained in the T3 treatment and lowest protein contained 5.827% at T0. The highest proteincontained 7.233% at treatment L4 5.982% and the lowest protein contained in L1 treatment.

Keyword : Cassava, Moringa leaves, Mocaf, Cookies

## 1. Introduction

*Cookies* is a result of roasting food products made with the basic ingredients of flour, with a final moisture content of less than 5%. Typically formulation enriched biscuits made with additional ingredients such as fat, sugar and material developers. Quality *cookies*other than specified by its nutritional value is also determined by the color, aroma, taste, and kerenyahannya. Crispness is a very important quality characteristics for receipt of dried product. Crispness is determined by the protein content in the form of gluten flour used [1].

Flour mocaf (Modified Cassava Flour) in Indonesian called cassava flour is modified, said to be a modification process because in the manufacture mocaf do special process called fermentation or curing using the services of microbes or certain enzymes, so that during the process of fermentationoccurs changes outstanding in both sweet period of change aspects of physical, chemical, and microbiological and sensory. Mocaf has characteristics similar to wheat so that it can be used as a substitute for wheat flour or a mixture of 30% - 100% and can reduce the cost of wheat flour consumption of 20- 30%. Mocaf have a little protein made from wheat flour, while rich in protein, carbohydrates richer mocaf [1].

Proteins derived from plants so far only known to be obtained from nuts is equal to 23-35 grams of protein per 100 grams of nuts. But besides nuts No other plant protein content is high compared to other types of vegetables, which is a smaller place. In 100 grams of Moringa leaves contain 6.7 grams of protein and moringa leaf powder contains 27 grams of protein [1].

Currently the modification of cassava flour to substitute wheat flour have been developed. Cassava flour that has been modified by treatment with fermentation has characteristics similar to wheat but has a lower protein content. Therefore, the authors are interested in doing research with the title "The addition of Moringa leaves and Old

Flour Baking Against the protein content in the manufacture of *cookies*from Mocaf (Modified Cassava Flour).

## 2. Material and Methods

*2.1. Materials Research*

Materials used are cassava (Manihot utilisima), leaves of Moringa (Moringa oleifera Lam), lactic acid bacteria (starter), wheat flour, eggs, blowing agents, salt, eggs, margarine and sugar.

*2.2. Research Methods*

The research uses Complete Randomized Design (CRD) factorial with (2) two replication. Factor I is addition of moringa leaf powder with password (T) which consists of 4 standard, namely : $T_0$ = 0%, $T_1$ = 3%, $T_2$ = 6%, and $T_3$ =9%. Factor II is long roasting with password (L) which consists of 4 standard, namely : $L_1$ = 5 minute, $L_2$ = 10 minute, $L_3$ = 15 minute, and $L_4$ = 20 minute.

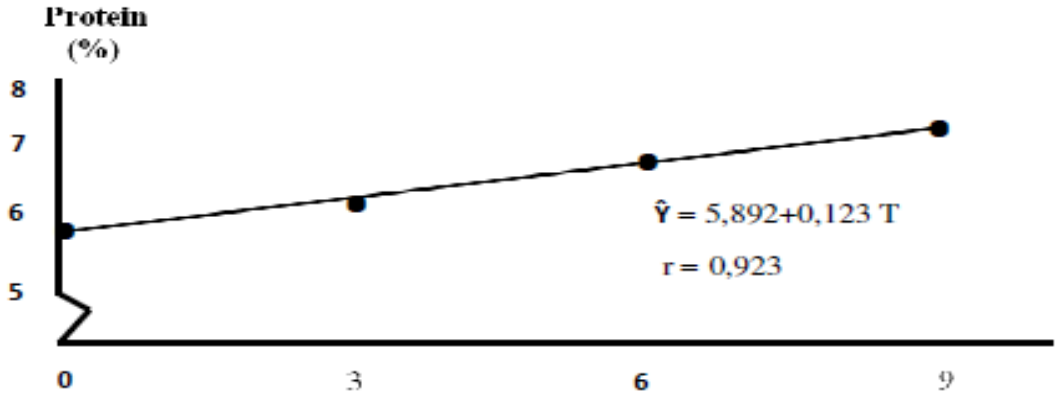
*2.3. Implementation Research*

1. Combine ingredients mocaf flour plus wheat flour in the ratio of 1: 1 and then coupled with moringa leaf powder in accordance with 0%, 3%, 6% and 9% respectively - each 100 grams of material, then add 14% egg yolk, salt 3%, sugar 10%, 30% margarine and materials developers 0.5% that has been stirred using a mixer for 15 minutes, then after being printed by using a mold dough*cookies*.
2. Then the roasting is done in accordance with the treatment of 5 minutes, 10 minutes, 15 minutes and 20 minutes respectively at a temperature of 1800C
3. Once cooked analysis of protein content.

Table 1. Effect of addition of Moringa Leaves Against Wheat Protein Ingredients

| TREATMENT (T) | MEAN (%) | RANGE (P) | LSR | | NOTATION | |
|---|---|---|---|---|---|---|
| | | | 0,05 | 0,01 | 0,05 | 0,01 |
| $T_0$ = 0% | 5,827 | - | - | - | CD | CD |
| $T_1$ = 3 % | 6,483 | 2 | 0,533 | 0.734 | BC | BC |
| $T_2$ = 6 % | 6,593 | 3 | 0,559 | 0,771 | B | B |
| $T_3$ = 9 % | 7,096 | 4 | 0,574 | 0,791 | A | A |

Description: The letters different in notation column showed a significantly different effect on the level of 5% and highly significant at the 1% level.




The addition of Moringa Leaf Flour (%)

Fig.1. Moringa Leaf Flour relationship Addition to Protein

*3.2. Effect of long Roasting on Protein*

Table 2. Effect of long Roasting on Protein

| TREATMENT (L) | MEAN (%) | RANGE (P) | LSR 0,05 | LSR 0,01 | NOTATION 0,05 | NOTATION 0,01 |
|---|---|---|---|---|---|---|
| $L_1$ = 5 MENIT | 5,982 | - | - | - | CD | CD |
| $L_2$ = 10 MENIT | 6,092 | 2 | 0,533 | 0,734 | BC | BC |
| $L_3$ = 15 MENIT | 6,492 | 3 | 0,559 | 0,771 | B | AB |
| $L_4$ = 20 MENIT | 7,233 | 4 | 0,574 | 0,791 | A | A |

Description: The letters different in notation column showed a significantly different effect on the level of 5% and highly significant at the 1% level.

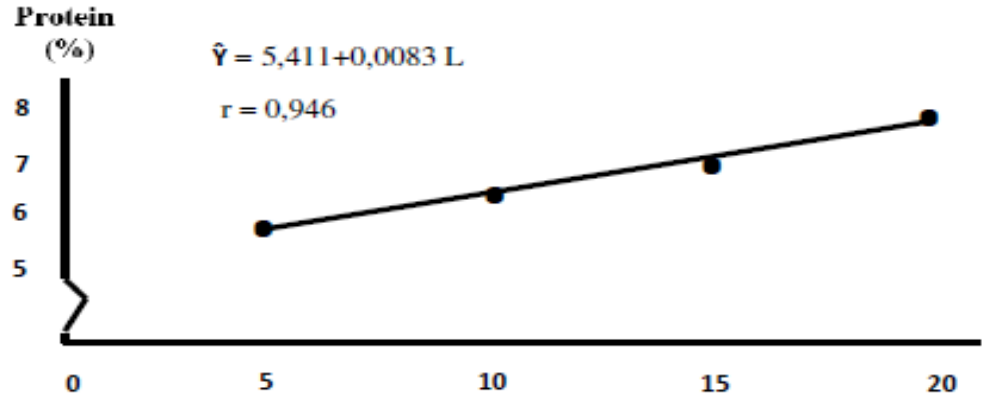



Fig.2. Relationships long Roasting against Protein

## 3. Result and Discussion

*3.1. Effect of Addition of Moringa Leaves Against Wheat Protein Ingredients*

From Figure 1 we can see that the higher the addition of Moringa leaf powder, the levels of protein produced will increase. This is due to the high levels of protein in the processed food due to the use of Moringa leaves are high in protein[10], The use of flour of Moringa leaves can be said to be successful in increasing levels of protein biscuits and can be used as an alternative to high-protein food for children and adults.

From Figure 2 it can be seen that the longer roasting the protein content increased. This is because the longer the roasting is used to mean the possibility of reduced material to the greater water content, so that the material in a dry state and can increase levels of a protein. This is consistent with the statement Desrosier (2006), states that during the roasting process food loses water content, which resulted in higher levels of nutrients in the mass remained.

The amount of protein and carbohydrates are present per unit of weight in the dry food will be greater than the freshest ingredients. This is what causes the protein content increased in every long process of roasting different.

**Conclusion**

Based on the results of research and discussion it can be concluded :

1. The addition of moringa leaf powder gave highly significant effect (P> 0.01) to the protein content.
2. Long roasting gives highly significant effect (P> 0.01) to the protein content.
3. The highest protein 7.096% contained in the T3 treatment and lowest protein contained 5.827% at T0. The highest proteincontained 7.233% at treatment L4

5.982% and the lowest protein contained in L1 treatment.

## Reference

[1] Desroisier, 2006. Technology Of Food Preservation. AVI Publishing Company Inc. Translated by Muchtadi Muljoharjo. Food Preservation Technology . University of Indonesian Press. Jakarta.

[2] Matz, 1991. Cookie. AVI. Co. Inc., Westport. London

[3] Nugroho, 2006. Optimizing Utilization of Fish and Sweet Potatoes White pepetek For Partial substitution Flour In Manufacture Confectionery.Thesis Department of Aquatic Product Technology, Faculty of Fisheries and Marine Science, Bogor

[4] Salim, E., 2011. Cultivate cassava (Manihot utilisima) Being Flour Business Mocaf Alternatives Food Products Flour Substitute . Lily publisher. Yogyakarta.

[4] Tarau. 2011. Composition in Processing Flour Confectionery. University of Gajah Mada. Yogyakarta.

# MEDICINES, PUBLIC HEALTH, ENGINEERING AND NATURAL SCIENCES

# Implementation of Hospital Information System in RSUP Dr. M. Djamil Padang 2016

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## Abstract

Background: Implementation of information systems in hospitals has created to improve its health systems for the benefits of its people. RSUP Dr.M.Djamil Padang, as the biggest public hospital in West Sumatra District, has started to realize the necessity that was called "Enterprise Hospital System". This study aims to analyze the implementation of hospital information system in RSUP Dr.M.Djamil Padang. Methods: This was qualitative study with depth interview, observation, and document review. This study conducted in the General Surgery Unit, RSUP DR.M.Djamil Padang with 6 informans. Data were analyze by content analysis. Results: The study showed that there were operators who not suitable with the standard of qualification. As a result they couldn't solve if some error in the software and hardware happened at the implementation of hospital information system. They have to asked the Hospital Management Information System Unit for help then took more time to collect and input patient data. Conclusion: It is suggested to make training to improve the quality of all operators. Guidelines based on the need of each units needs to help operators solve the software and hardware problems.

Keywords: hospital information system, health information system, implementation information system

## 1. Introduction

The need of data and information currently developing very rapidly, in terms of quantity and quality. The enactment of Law no 14 of 2008 on Public Information Openness (KIP) makes the availability of data and information is absolutely needed by public service agencies like hospital. Data and information is annually changed through times, so that the revision in the Hospital Information System that already exists today is absolutely necessary.(1)

Every hospital have to perform registration and reporting about all organized activities deal with hospital in form of Hospital Management Information System (SIMRS)(2) Hospital

Management Information System is an integrated information system prepared for handling whole process of hospital management process, starts from system service and action for patient, medical record, pharmacy, pharmacy warehouse, collection, personnel database, distribution of employee salary, accountancy process until the management control. (3)

Management Information System is very important for hospital development. The final result of hospital effort can be measured by some indicators, like medication services or administrative indicator for example hospital's financial and management information system which give a description of success and failure, including quality aspect like satisfaction and safety of patients. The fast changing of society, and also the fast changing of technology of medication tools, makes program or needs have to follow and

fit them. Because of this program evaluation, information will play important rule for management and for decision making in order to do adaptation.(4)

Evaluation toward implementation of Hospital Management Information System (SIMRS) must have done because evaluation will value or measure the benefits which is gotten from SIMRS implementation and to find potential problems which were facing by users and hospital. The result of evaluation can be used as reference to repair or to make SIMRS perfect and also to develop existing potency, so that SIMRS will be better, perfect and also is able to support goals, vision and mission of hospital(5)

The circumstances of Installation of Management Information System (MIS) task are including outpatient, the hospitalizations, IGD, service of outpatient, the hospitalizations, IGD, supporting services (laboratory, IDT, radiology, IRM,*radiotherapy*), payment, BPJS claim, network relocation, *hardware* repair, *up-grade, and* application, network and *hardware maintenance.*(3)

Based on early survey which is done through an interview with Head of Installation of Management Information System (SIM) of RSUP Dr.M.Djamil on 22nd February, 2016 there are problems that still exist in implementation of Management Information System (SIM) in RSUP Dr.M.Djamil. The problems are there are disturbances that occur from *hardware, software,* as well as network in every working unit in RSUP Dr.M.Djamil. These things cause disturbance on patient's services.

Disturbances that are found in General Surgical Unit Polyclinic are *software* disturbances like *error* application. This causes medical record operators in General Surgical Unit Polyclinic have to report it first to responsible party in Management Information System (SIM) and have to wait the handling of disturbance until it is fixed. The duration that is taken for the handling of disturbances takes quite long time, about 30 minutes, so it causes disturbance on patient's service.

The problems of hardware and network are also found in SIM RS implementation. Hardware disturbances like out of order printer and monitor, and if there is no available back up *hardware* takes time about one day to repair it. Disturbance on network like network that is suddenly disconnected so it obstructs registration process and services to patient.

Problems that happen in General Surgical Unit Polyclinic causes long duration of service waiting time that is given to patient. Waiting time is an *output* or result of implementation of management information system especially in registration side which can influence utilization number in the future. Registration activity is a early reflection in making image in hospital which is will be a measurement in defining patient's satisfaction.(6)

Besides that, in handling hardware and software disturbances must be treated and finished quickly because it will affect patient's service and patient's satisfaction.(3)

Based on the explanation of problems above, the researchers are interested to analyze implementation of Management Information System (SIM) of General Surgical Unit Polyclinic in RSUP.Dr.M.Djamil Padang in 2016.

## 2. Methods

The kind of research that is used in this research is qualitative. The subject of this research is Management Information System (SIM) in General Surgical Unit Polyclinic in RSUP.Dr.M.Djamil Padang. Defining informan technique that is used in this research is *purposive sampling.* Informan in this research is Head of SIM Instalation, Head of General Surgical Unit Polyclinic, *Software System Support , Hardware System Support ,* Network *System Support,* and Data Entry

Operator (Medical Record) Surgical Unit Polyclinic in RSUP Dr.M.Djamil. The technique of collecting data is *Indept Interview*, observation and document study. Data analisis uses content analysis. Data validation uses resources triangulation and method triangulation.

## 3. Result and Discussion

### *3.1. Result*

The executing peoples who are engaged in Management Information System (SIM RS) in General Surgical Unit in RSUP Dr.M.Djamil are an installation operator of SIM RS in *system support software, system support hardware,* and network *system support* and Data Entry Operator (Medical Record) Surgical Unit Polyclinic division. Based on Indept observation and interview, known that the amount operators in *system support software* 5 people, *system support hardware* 3 people, network *system support* 2 people, and medical record operator of General Surgical Unit 1 people.

*SIM RS and whole working units which are integrated with system are engaged, generally from medical record..".(Inf-1)*
*"In here yaa there is only medical record operator" (Inf-2)*
*".... the software here are in portion, accidentally maintenance is divided by buk dewi for the hospitalizations 2 people, outpatient 1, supporting 2, IGD 2." (Inf-3)*
*"In hardware there are 3 people. "(Inf-4)*
*"There are 2 people here managing network"(Inf-5)*
*"..... only me in Medical Record" (Inf-6)*

Competencies that are owned by operator in Management Information System (SIM) is seen from educational side for Management Information System (SIM) Instalation consists of S1 in Computer, S1 in Economics, D3 in Computer and Senior High School. The last education of Entry data operator (medical record) General Surgical Unit Polyclinic is D3 in Medical Record.

Based on the number of operators that are engaged in implementation of Management Information System (SIM), SIM RS instalation can fix disturbance which comes from various working unit well. Problems that are existed in general surgical unit polyclinic are entry data operator (medical record) experiences difficulty in performing work alone if the patients who comes are quite many.

The related coaching deals with Management Information System (SIM) which has been done does not include all operators that are engaged. The coaching that is done like coaching about application that is used which has target on entry data operator (medical record) from related working unit and Management Information System (SIM) operator *system support software* division. The coaching that supports another working sectiosn like *system support hardware* and network *system support* have not been held.

Based on indept interview and document study, it is known that there is no manual book that gives instruction in implementation of SIM RS like written manual. Manual that already there is user guide which gives to coaching participants when coaching of Management Information System (SIM) was held.

Manual book of that only shared with participant of coaching, including medical record office in every related working unit. In the implementation, it can be seen that the manual books have not shared fairly because the medical record operator in General Surgical Unit does not know that there is manual book of Management Information System.

The implementation of Management Information System (SIM) in RSUP Dr.M.Djamil is using *Enterprise Hospital System* application. Based on indept interview known that there are problems still exist in the

form of disturbances which happen in the field. The implementation of this application has not spread all over hospital, but for all related working unit which directly connected with patient's service has performed this application. It causes because the application is still in developing phases.

Application that is being used now is still in changing and repairing according to the need of hospital. The reason of changing are the changing of policy or because to adapt with field condition. There is no frequency of defining times in doing change and improvement of this application, it is only done in defining times according field's condition.

The implementation of SIM RS in RSUP Dr.M.Djamil is done by using LAN (Local Area Network) computer technology which covers all division of hospital. There are still disturbances in network which supports SIM RS implementation. The disturbance which often happens are like suddenly disconnected network, so that it can blocked service process to patient.

The problem on network in implementation of Management Information System (SIM) often happens. It caused by the disturbance of main server, per segment disturbance and disturbance in related working unit, like the cable which is being stepped on untentionally so that network becomes disconnected. According to indept interview with medical record operator in General Surgical Unit Polyclinic, explains that the impact of disturbed network can disturb services that gives to patient.

The supporting tools in implementation of Management Information System are computer equipment that are distributed to all division of Hospital in 179 unit. There is 1 computer ware that is used in General Surgical Unit Polyclinic to implementing Hospital Management Information System (SIM RS). If it seen from its quantity in totality has fit with hospital need and can help service process. Based on indept interview result, from its quality is not good enough, because there is disturbance in its using.

In supporting implementation of Management Information System (SIM), there are still disturbances in *hardware* like in monitor, printer, keyboard, mouse and another tools. The average of disturbances or damage happens about 3 times a day. If there is damage in related working unit deals with *hardware* so that working unit reports it to SIM RS Installation division, and SIM RS operator's *system support hardware* division is directly going to field to see the problematic hardware condition.

The process of collecting data in implementation of Management Information System (SIM) is performed by medical record operator in every working units. The whole entry data process is already *online* but when there is disturbance, it is performed manually. In collecting this data, *software*, *hardware*, and network took important role because if there is disturbance, it will block that collecting data process.

*"...In collecting data in context data entry it is already all online..." (Inf-1)*

*"....Software takes important role, if there is no software of reports to management and core that are using software" (Inf-3)*

*"...Hardware takes important role, if computer is broken it will be disturbed " (Inf-4)*

*"...Network takes important role, because the server of whole computer is here, so data input and storage need network" (Inf-5)*

*"...Everything is online, but if there is problem, it will be manual" (Inf-6)*

There is no processing process in implementation of Management Information System (SIM) which is done by medical record operator in every working unit. Based on indept interview, the data that is inputted is directly saved in main server of SIM RS installation. The saving process of data in

implementation of Management Information System (SIM) is performed by every working unit related with SIM RS installation. The data saving is done after the data input process and data is being saved in main *server*.

The controlling data process in implementation Management Information System (SIM) has been done, which is controlling that is performed by Management Information System (SIM) installation party to all related working unit. Besides that, there is a controlling that is done by financial director as a leader of SIM RS installation who directly controls the implementation of SIM RS in hospital if it is already run well or have not.

Based on indept interview, it is known that *front office* working unit gives direct service to patient which are already integrated with SIM RS. Nonetheless, in General Surgical Unit, there are problems that still exist in term of *software, hardware,* and network in its implementation.

The impact of Management Information System (SIM) in whole way gives ease in work because SIM RS is online so that the hospital management process is already integrated between one division and another division. From the timing side, it is more effective and efficient, and also more accurate in collecting and saving data. But if there are disturbances in *software, hardware* and network will block service process to patient so that the waiting time of patient will be longer and cause the lack satisfaction of patient.

*"...the first one is function of SIM RS gives ease, then the timing is more faster than manual, then the data is more accurate..." (Inf-1)*
*"...if there are no problems smooths patient service because everything is online, there is no more manual" (Inf-2)*
*"The impact of reporting side, patient data saving, the registration of patient in kind of Outpatient, The Hospitalizations, IGD will be more accurate..." (Inf-3)*
*"Makes work easy..." (Inf-4)*
*"...Gives ease, and more accurate data, and more faster too…"(Inf-5)*
*"Sometimes makes easy, sometimes if there are problems it will make patient confused, the impact is blamed on patient, for example if patient has double data, the patient satisfaction will be decreased..." (Inf-6)*

*3.2. Discussion*

The human resources or operator that are engaged directly with RSUP Dr.M.Djamil's Management Information System (SIM) in General Surgical Unit Polyclinic are SIM operator in *system support software, system support hardware,* and network *system support* division. There is *a system support hardware* operator who has background in S1 in Economics, but the standard of education which is already agreed is S1 in Computer. Besides that in network *system support* there is operator who has education background in Senior High School meanwhile the education standard which already agreed must be from D3 in Computer.

According to the result of research that has been doned by Yunides about analysis of Implementation of Hospital Information System in Supporting Arranging Planing in RSUD Lubuk Basung Agam Regency in 2008. In that research the problem that is found is lack of executing operator so it causes double task for defined operator who prefers to give priority in giving service to patient[7]. Meanwhile, Sari's research is about of analysis of SIKDA implementation in Padang of 2011 describes SIK managing operator in Dinkes of Sumatera Barat province is already adequate and have ever follow coaching about SIK so there is no double task in task implementation. [8]

Related coaching of Management Information System (SIM) is already performed but the target of coaching has not cover all related operators that are engaged. The coaching that has bee done like coaching about application with data entry operator from working unit related with Management Information System (SIM) and Management Information System (SIM) *system support software* division as target. Coaching which supports another work division like *system support software* and network *system support* have not held.

Method that has been seen from research is related with manual book or manual of Management Information System (SIM) implementation, policy that organizes implementation of Management Information System (SIM), Standard Operational Procedure (SOP), and the report of result implementation of Management Information System (SIM) in RSUP DR.M.Djamil. The manual book that is given to entry data operator (medical record) as manual that contains instruction of implementation of Hospital Management Information System (SIM RS) is not there yet.

The existing manual book is in form of *user guide* that is given to coaching participant when coaching of application that is used was held. Participants of that coaching are medical record operators who act as *user*. Based on indept interview with entry data operator (medical record) General Surgical Unit Polyclinic have not follow coaching so that she or he does not have that *user guide.* This proves that not all medical record operators in RSUP Dr.M.Djamil have already follow given coaching.

Policy or procedure that organize the organization of Management Information System (SIM) in RSUP Dr.M.Djamil is in form of Standard Operational Procedure (SOP). The installation of Management Information System (SIM) has Standard Operational Procedure (SOP) which is valid for implementation of Management Information System (SIM) in every working unit in RSUP Dr.M.Djamil. Written document about Standard Operational Procedure (SOP) contains submission of *software* improvement, submission of additional *software, troubleshoot* handling, the change of tariff setting, submission of *hardware* improvement and *hardware* additional submission and relocation. Standard Operational Procedure (SOP) related with that Management Information System (SIM) is only owned by SIM RS installation and not distributed to every related working unit. That Standard Operational Procedure (SOP) of Management Information System (SIM) is issued in 1st of May, 2015 and already settled by Main Director of RSUP Dr.M.Djamil.

The factors that have important roles in the success of implementation of Management Information System (SIM) is technical factors consist of *hardware, software,* and network factor. In general, facility and tools are supporting tools of an effort that is done in public service, because if those two things do not available so that all activities that are done can not be achieved befit with plan.

Machine like hardware that supports implementation of Management Information System (SIM) in RSUP Dr.M.Djamil especially General Surgical Unit Polyclinic. From quantity side, hardware that is distributed to hospital especially General Surgical Unit Polyclinic is already befit with the need. From the quality side, it is still not good enough because the related problem with hardware often happens like disturbance on printer in General Surgical Unit Polyclinic.

Based on research that has been done by Bulqis about Implementation Analysis of Management Information System in Support Arranging Planning RSUD Prof.DR.Hanafiah SM Batusangkar of 2009 declares that the tools that is needed to process data about Hospital Management Information System

already exists but there is lack of computer condition which is still not good enough.[9]

The process of collecting data in implementation of Management Information System (SIM) is done by data entry operator (medical record) in every working units and data entry process are done online. If there are disturbances from the software, hardware, or network side so data recapitulation is done manually. The collecting data process is a very important process in implementation of Hospital Management Information System (SIM RS) because data that is collected must be precise and accurate so that service process of patient can be done well. In this collecting data process, software, hardware, and network play very important roles.

The processing of data is a process to change data meaning to information and can be used in supporting various management activity including decision making activity. The change of form and meaning are critical because the data actually do not have intrinsic value to decision making. The thing that has critical value is only information. It means that the processor of data has to understand the intrinsic difference between data and information. The one way that can change form and meaning of data into information is with data analysis so that there is only one interpretation that is faced.

The data that is inputted in every working unit are being saved in main server of installation of SIM RS. That data are directly saved in main *server* and are not saved in every related working unit. So, if there is disturbance of server there is no back up data that are saved in another division, because all data are saved in that server only. The output of data processing are information that have to be saved well so that its security is guaranteed, efficient in cost and from third point of view is not used by another people who are not responsible and do not have right on it, safe from damage because of not proper saving place and saving way, safe from fire.

The data control of Implementation of Management Information System (SIM) in RSUP Dr.M.Djamil is done by various party. The control of Management Information System (SIM) installation is done by Financial Director as responsible person of implementation of Management Information System (SIM). Based on that control it can be known that if there are disturbances in implementation of Management Information System (SIM) that are caused by software, hardware, network or the neglect of operators. Meanwhile the control of General Surgical Unit Polyclinic is only can be done by the Head of General Surgical Unit Polyclinic in implementation of Management Information System (SIM) in that General Surgical Unit Polyclinic.

The implementation of Management Information System (SIM) in RSUP Dr.M.Djamil for all front office working units have been integrated with system. Nonetheless, there are disturbances exists in every working unit in software, hardware and network. Especially in implementation of General Surgical Unit Polyclinic, the disturbances are in software and network side. This disturbance causes collecting data is not on time and add waiting time of patient and the decrease of patient's satisfaction in patient service process.

Based on indept interview, it is known that the impact of implementation of Management Information System (SIM) in all over gives ease in work because implementation of online SIM RS so that hospital management process is integrated between one division and another division. If it seen from timing side, it is more effective and efficient, and collecting data and saving data will be more accurate. Nonetheless, if there are disturbances in software, hardware and network, will block service process to the patient so it causes waiting time of patient becomes longer and the satisfaction of patient will be decreased.

## Conclusion

The number of operators in SIM RS installation still not befit with the need and standard of education that is already settled. The coaching that is given have not include all operators that are engaged in SIM RS implementation. Implementation method of SIM RS does not have manual books that are distributed to every related working unit. The material that supports implementation of SIM RS are software and network that are still founded in disturbed way. Machines that support the implementation of SIM RS have already befit with the need of quantity side but there are disturbances in form of damage on printer and monitor. The collecting data are done by data entry operator (medical record) in every related working unit in *online* way. The processing of data is done directly by the system so that the data that are served are in form of table and graphic. The data entry in every related working unit is done by SIM RS installation, and the control of SIM RS installation is done Financial Director.

It can be suggested that the number of SIM RS installation operators befit with the need and education standard, all coaching that are done can be participated by the executing operators, the making and distribution of manual book and distribution of SOP to all related working units can be done, and good controlling of implementation of SIM RS especially in General Surgical Unit Polyclinic.

## References


1. Kementrian Kesehatan RI. Juknis Sistem Informasi Rumah Sakit. Jakarta: Direktur Jendral Bina Upaya Kesehatan; 2011.
2. Kementrian Kesehatan RI. Undang-Undang Republik Indonesia No. 44 Tahun 2009. Kepmenkes RI; 2009.
3. Instalasi SIM RS. Pedoman Pelayanan Instalasi Sistem Informasi Manajemen RS. Padang: RSUP DR.M.DJAMIL; 2015.
4. S.Sabarguna B. *Buku Pegangan Mahasiswa Manajemen Rumah Sakit*. Jakarta: Sagung Seto; 2011.
5. Sabarguna B, Iyus Suhandi. *Buku Pedoman Mahasiswa Sistem Informasi Manajemen Bidang Kesehatan*. Jakarta: Universitas Indonesia; 2011.
6. Aditama TY. *Manajemen Administrasi Rumah Sakit*. Jakarta: UI-Press; 2003.
7. Yunides. Analisis Pelaksanaan Sistem Informasi Rumah Sakit Dalam Mendukung Penyusunan Perencanaan di RSUD Lubuk Basung Kabupaten Agam [SKRIPSI]. Padang: Universitas Andalas; 2008.
8. Sari AF. Analisis Pelaksanaan Sistem Informasi Kesehatan Daerah (SIKDA) Kota Padang Tahun 2011 [SKRIPSI]. Padang: Universitas Andalas; 2011.
9. Bulqis. Analisis Pelaksanaan Sistem Informasi Rumah Sakit Dalam Mendukung Penyusunan Perencanaan di RSUD Prof.Dr.Hanafiah SM Batusangkar tahun 2009. Padang Universitas Andalas; 2009.

# Implementation Patient Safety Standards in Basic Emergency Obstetric Care Community Health Center (BEOC_CHC) Padang

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## Abstract

A high number of Adverse Event based on the data from Ministry of Health RI showed that patient safety still not going well, while patient safety is a measure of the quality of health services in Indonesia. The purpose of this study is to determine the implementation of the seven standards of patient safety as the basic of patient safety model development based on Malcolm Baldrige in BEOC -CHC of Padang as the implementation of maternal and child safety. This study uses a qualitative research method and the number of informant is 25. The data collecting was done by in-depth interview and Focus Group Discussion that will be written on the transcript in the form of matrix and analyzed through resource and method triangulation. Based on the study result, there is no guidelines about patient safety from Department of Health of West Sumatra to the BEOC -CHC, it is also obtained that the patient safety incident that occurs is in the form of patient falls, diagnostic errors and drug delivery personally without reporting to the Department of Health. Out of seven standard of patient safety, only the third standard that has been accomplished. It can be concluded that the patient safety hasn't been completely implemented, so it is necessary to develop Malcolm Baldrige-based patient safety model that is suitable to improve patient safety in BEOC -CHC

***Keywords*:** Patient Safety, BEOC-CHC, Standard

## 1. Introduction

WHO had identified the risk of adverse event in health services which are serious and threaten the safety of patients globally? [1] Risks detected since the report of the Institute of Medicine (IOM), reported adverse events on the hospital in Utah and Colorado by 2.9% which 6.6% of them died, and the New York Hospital by 3.7% with the mortality rate is 13.6% of them [2-5] The report of adverse event in Indonesia from the Ministry of Health is quite high. Until February 2016, it is reached 289 reports. The type of adverse event consists of 69 events of near miss (43.67%) in the form of medication errors (29.2%), patient falls (23.4%), canceled operations (14.3%), diagnosis errors (11%), incorrect laboratory tests (8.4%) and incorrect roentgen (5.2%). [6].

Since the implementation of JKN in 2012, PT BPJS as the executor of JKN adjust a procedure starting from the first level of health care. To reduce maternal and infants mortality rate in Indonesia was done by the

availability of BEOC-CHC in each district as a gate keeper for the safety of mothers and children. BEOC-CHC will improving access to maternal and neonatal to cope with obstetric and neonatal emergency cases which is the largest contributor to maternal and child mortality rate [7]. The aspects of patient safety in the primary health centers appears as the part of the Regulation of Ministry of Health number 75 of 2014, but still no clear guidelines for its implementation [8, 9].

There isn't much information about adverse event in primary care [10]. Based on some research, it is in the form of missed or delayed diagnosis and medication management [11], medication and diagnosis errors, failures communication between the human resources [12] Teamwork, management support, communication, staff and the value of patient safety. [13, 14], the lack of guidance, ineffective communication, lack of knowledge and lack of quality assurance mechanisms [15].

To provide a high quality health services and be able to compete in the global marketplace, it can be used the Malcolm Baldrige Criteria for Performance Healthcare (MBHCP). The advantages of MBHCP are its ability to provide a comprehensive and integrated assessment. MBHCP is used because of its ability to identify the strengths and opportunities for improvements, provide a framework to improve performance advantages by giving liberties to the management to implement its management strategies. An integrated management framework includes every factor that defines the organization, operational processes and a clear and measurable work, increases the process speed and quality of work, building a high work system, translating the vision and mission into strategy and builds the loyalty of patients. [16] The purpose of this study is to determine the implementation of the seven standard of patient safety as the basic of patient safety model development based on Malcolm Baldrige in BEOC-CHC of Padang as the implementation of maternal and child safety.

## 2. Material and Method

This study uses a descriptive exploratory study with a qualitative design. The study was held in Department of Health of West Sumatra as the policies holder, Department of Health of Padang as the direct supervisor and BEOC-CHC. The study was conducted from January to August 2016.

The number of informants is 25 wich is the Head of Health Registry Section, Accreditation, and Sertification of Department of Health of West Sumatra, the Head of Health Services of Department of Health of Padang, the Head of Lubuk Buaya and Seberang Padang BEOC-CHC. All informants got in-depth interview. Six health personnel from Lubuk Buaya BEOC-CHC, nine health personnel Seberang Padang BEOC-CHC, and also 12 patients got focus group discussion (FGD). Informants get asked about the implementation of the seven standards of patient safety based on a system approach in terms of input, process and output using the guidelines which are derived from the hospital patient safety guidelines which were modified and adjusted based on research purposes.

The result of in-depth interviews and FGD will be written in the field notes, personal documents, official documents, drawings and photographs. Furthermore, the result would be read and analyzed. The analyzed was done by interpret and dechiper the data that has been acquired into a substantive theory. The data that had been required were analyzed descriptively, summarized and presented.

## 3. Result and Discussion

Based on the 25 informants in this study, the average age of the respondents is 39 years old, with the youngest is 25 and the oldest is 52 years old. The average length of

work is 13 years, the longest is 22 years and the shortest is 2 years. For almost all patients were housewives.

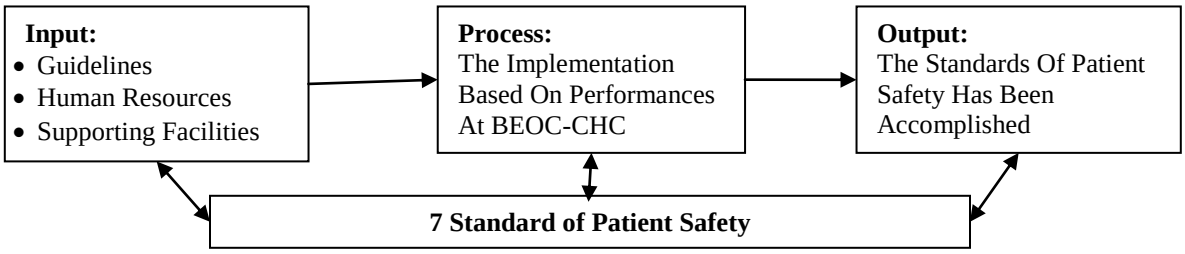


Figure 1. Thinking Flow of The Implementation of Patient Safety Standard in BEOC Health Centers

Based on the results, the adverse event was found last year in BEOC-CHC is medication errors and patient falls out of the bed. This adverse event was completed amicably between health centers and patient's family. Incidents didn't report in writing to the Department of Health of Padang because there are no guidelines and reporting format for adverse events. Until now there are still no guidelines for patient safety from Department of Health of West Sumatra, Department of Health of Padang and BEOC-CHC in Padang about the implementation of patient safety in the BEOC-CHC.

*3.1. Patient Rigths*

There are no guidelines to fulfill the patient rights on BEOC-CHC yet. The doctor in charge of service making plans of service and done assessment of patients. Medical records and informant consent as the document of services planning and implementations. In the implementation process, information provide and explanations to the patients and their families about plans and results are not always given, but for every services performed is always preceded by the signing of informed consent. Patients or family explained about the services that will be given but without being informed about the result of services, the further services plans and the likelihood of adverse events. The output has not been running well. Patients have not been informed about the results of the services given and the possibility of adverse events.

*3.2. Educate Patients And Their Families*

There is no spesific plans to educate the patients and their families about patient safety at BEOC-CHC. The health guidance and promotion about the maternal and child safety limitary given only to the mother through maternal classes every month, without educate the patient's family. The implementation of giving right informations, transparent and honest to patients was not done yet but patients have already know their obligations and responsibilities, patients understand and accept the consequences of services, also patients and families fulfill their financial obligations. The output has not been accomplised too.

*3.3. Patient Safety And Continual Care*

Planning of the patient flow from registered until finishwas coduct through the workshops in BEOC-CHC every month. Every health personnel incharge from patients register until the medication and patient go home. Standard Operational Procedures (SOP) for each supporting facilities and the flowchart of patient services flows has been displayed on the walls of health centers. The coordination of services started from the patient registered until going home accordingly to the patient needs, but sometimes constrained due to the limitations

of doctors, sometimes patients were examined and given a prescription not by doctor. The improvements of communications and transfer comunication among health personnel running well. The output already performing well.

3.4. *The Use of Improvement Methods of Performances to Evaluate and Improve Patient Safety*

Planning in the input by conducted performance assessment for health personnels is carried through the accreditation process of BEOC-CHC. All heath personnel of BEOC-CHC will empowered all health personnels. Lubuk Buaya BEOC-CHC is one of the best CHC in Padang which already have an ISO 9001 certificate. Implementation of patient safety not done yet. There is no designing plan of improvement with the 7 standards patient safety, accumulation of data, such as incident reports, risk management and audit quality of health services. Likewise, there has been no intensive evaluation of adverse events and proactively evaluate the high-risk cases, because there are no result of data analysis, thus the change of system have not been implemented. The output has not accomplished.

*3.5 The Role Of Leadership To Improve Patient Safety*

For input, the planning has not spesifically for patient safety. The head of BEOC-CHC has not appointed a specialized team. There are no guidelines and documents about patient safety in the health center yet because the patient safety issue is still a new issue for the health center. The implementation is also has not been done yet, no interdisciplinary team, no risk identification, no mechanism of work, no responsive procedure towards incidents, no internal and external reporting mechanisms, no mechanism to handle incidents, no open collaboration and communication between units, no resource and information system, no measurable targets. The output has not been accomplished too.

*3.6. Educate the Personnels about Patient Safety*

Plans for training and orientation process about patient safety which adjusted based on the decision from the Department of Health of Padang as the direct supervisor of BEOC-CHC. There are no guidelines and documents about patient safety. There are no integrating patient safety topics in every in-service training activities and also providing a clear guidance about reporting incidents yet, but there are trainings about teamwork to support interdisciplinary approach and collaboration in serving patients. Based on the output, education and training programs and orientation about patient safety for new health personnels in accordance with their respective duties has not yet accomplished,

*3.7. Comunication Is The Key To The Personnels To Achieve A Patient Safety*

There is no spesific planning about comunicating about patient safety. There are no guidelines and documents about patient safety in BEOC-CHC yet because the patient safety issue is still a new issue. For process, no budget avaiable to plan and design data proccesing to obtain data and information that is related to patient safety. For the output the implementation of data transmission still is not clear and the information still is not timely and accurate yet.

Based on the research, it is obtained that the adverse event occurred at BEOC-CHC at the last year. The implementation of patient safety in BEOC-CHC had been varied because no specific guidelines avaiable yet. [4]. In Indonesia, the regulation about the accreditation of primary health centers just had been declared recently on Permenkes number 75 of 2014, the implication in the health centers is not clear yet. [8]. The risk management which is the core of the implementation of patient safety in the

BEOC-CHC hasn't been running yet. And the adverse event still is the fault of the individual because 'blaming culture' still is applied. [17, 18]

The implementations of the 7 standards of patient safety at BEOC-CHC are reviewed with the system approach of input, process and output. Almost at all standards about patient safety (input, process and output) is not accomplished yet. Only the third standard about patient safety and continual care are systematicly goes well. Changes happen in health care providers, now patient is an important aspect of the design of the health care and improving the quality of health services (patient centered care). [19] Patients have an important role in helping to achieve an accurate diagnosis, in deciding on the appropriate treatment, in choosing an experienced and secured provider, in ensuring that the right treatment is given, monitored and adhered to, and in identifying the side effects and take appropriate action.[19-21] Therefore, it is important to fulfill patients' rights and educating patients and families. Comunication between healths personnel in the unit are good but between units still worse. Worse comunication can lead to adverse event. [22, 23] Good comunication between heath personnel and with patient and family it's important for patient safety.

Performance assesment in BEOC-CHC all this time based on health personnel attendances. Planing performance assessments in BEOC-CHC can be carried through the accreditation process of CHC. Good health personnel performance can improve patient safety through fix inadequate work space, fullfill incomplete equipment, given adequate information from the health personnel, and fix busy and disorganized working environment is busy and disorganized.[24] Such as strong, unwavering leadership and open communication and action can improve patient safety [25] with encourage and ensure the implementation of the patient safety program through the implementation of "7 Steps to the Hospital Patient Safety".

Health personnel as the practicioner must understand about patient safety. Educate the health personnel about patient safety was the sixth standard of patient safety, done by planing training and orientation process about patient safety [18, 26, 27] Since training of health personnel is determined by the Department of Health of Padang as the direct supervisor of BEOC-CHC, it is necesarry to planing about training health all personnel about patien safety in BEOC-CHC. Comunication was the key to the health personnel to achieve a patient safety. Good communication between health personnel, between health personnel and patients can reduce adverse event. Open communication is necesarry too, open communication would make good patient safety culture. To make good patient safety culture it is necessary to developt a model thats fit to patient safety in BEOC-CHC which can be developt bases malcom baldridge performance.

## Conclusion

In general it can be seen that out of the 7 standards of patient safety based on patient safety guidelines by KPPRS, only the third standard about patient safety and continuous care that has been running systematically, started from input, process until its output, while the patient safety standard number 1, 2, 4, 5, 6, and 7 hadn't been accomplished yet. It is required a patient safety system that matches the conditions of the BEOC-CHC based on Malcolm Baldridge performance as the standard of the performance of the organization which is applied on the patient safety performance.

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## References

[1] R. Varnam, "Patient Safety: A Primary Concern?," in *Institute for Innivation and Imprevement*, ed, 2012.

[2] A. Findyartini, R. Mustika, E. Felaza, Herqutanto, E. S. K. Wardhani, D. Iryani, *et al.*, *Modul Pelatihan untuk Pelatih Keselamatan Pasien*: Kolaborasi Bidang Pendidikan Proyek P4K-RSP, 2015.

[3] L. T. Kohn, J. M.Corrigan, and M. S.Donaldson, *To Err is Human: Building a Safer Health System*. Washington: National Academy Press, 2000.

[4] S. G. Marchon and W. V. Mendes Junior, "Patient safety in primary health care: a systematic review," *Cadernos de saúde pública*, vol. 30, pp. 1815-1835, 2014.

[5] R. Varnam, "Patient Safety: A Primary Concern? In Institute for Innivation and Imprevement," 2012.

[6] Subdit Pelayanan Medis dan Keperawatan, "Evaluasi pelaporan E-Reporting Pelaporan Insiden Keselamatan Pasien RS Sampai Februari 2016," presented at the Workshop Keselamatan Pasien di Hotel Horizon Bogor 2-4 Maret 2016, Bogor, 2016.

[7] D. R. P. Rahmanita and A. Bachtiar, "Penilaian Kinerja Pelayanan Obstetri Neonatal Emergensi Dasar di Puskesmas Poned Tanah Sareal Kota Bogor dengan Pendekatan *Balanced Scorecard tahun 2014*," *Fakultas Kesehatan Masyarakat UI*, 2014.

[8] *Peraturan Menteri Kesehatan Republik Indonesia No 75 Tahun 2014 tentang Pusat Kesehatan Masyarakat*, 2014.

[9] *Peraturan Menteri Kesehatan Republik Indonesia Nomor 46 Tahun 2015 tentang Akreditasi Puskesmas, Klinik Pertama, Tempat Praktik Mandiri Dokter, dan Tempat Praktik Mandiri Doketr Gigi*, 2015.

[10] J. Kingston-Riechers, M. Ospina, E. Jonsson, P. Childs, L. McLeod, and J. Maxted, "Patient safety in primary care," *Edmonton AB: Canadian Patient Safety Institute and BC Patient Safety and Quality Council*, 2010.

[11] M. O'Rourke, "the australian Commission on Safety and Quality in Health Care agenda for improvement and implementation," *Asia Pacific Journal of Health Management*, vol. 2, p. 21, 2007.

[12] S. G. Marchon and W. V. M. Junior, "Patient safety in primary health care: a systematic review," *Cad. Saude Publica*, vol. 30, pp. 1815-1835, 2014.

[13] P. A. Molloy, "Examining the relationship between work climate and patient safety among nurses in acute care settings," 2012.

[14] S. L. Walston, B. A. Al-Omar, and F. A. Al-Mutari, "Factors affecting the climate of hospital patient safety: A study of hospitals in Saudi Arabia," *International Journal of Health Care Quality Assurance*, vol. 23, pp. 35-50, 2010.

[15] C. M. Pettker, S. F. Thung, E. R. Norwitz, C. S. Buhimschi, C. A. Raab, J. A. Copel, *et al.*, "Impact of a comprehensive patient safety strategy on obstetric adverse events," *American journal of obstetrics and gynecology*, vol. 200, pp. 492. e1-492. e8, 2009.

[16] I. Sadikin, *Malcom Baldrodge National Quality Award (MBNQA)*. Surabaya: Lembayung Central Indonesia, 2010.

[17] M. R. Chassin, "THE NEED FOR A PARADIGM SHIFT IN HEALTHCARE CULTURE: OLD VERSUS NEW," *High Reliability Organizations: A Healthcare Handbook for Patient Safety & Quality*, p. 3, 2016.

[18] V. Nieva and J. Sorra, "Safety culture assessment: a tool for improving patient safety in healthcare organizations," *Quality and Safety in Health Care*, vol. 12, pp. ii17-ii23, 2003.

[19] B. Johnson, M. Abraham, J. Conway, L. Simmons, S. Edgman-Levitan, P. Sodomka, *et al.*, "Partnering with patients and families to design a patient-and family-centered health care system," *Bethesda MD: Institute for Family-Centered Care*, 2008.

[20] C. A. Vincent and A. Coulter, "Patient safety: what about the patient?," *Quality and Safety in Health Care*, vol. 11, pp. 76-80, 2002.

[21] R. E. Davis, R. Jacklin, N. Sevdalis, and C. A. Vincent, "Patient involvement in patient safety: what factors influence patient participation and engagement?," *Health Expectations*, vol. 10, pp. 259-267, 2007.

[22] M. Top and S. Tekingündüz, "Patient Safety Culture in a Turkish Public Hospital: A Study of Nurses' Perceptions About Patient Safety," *Systemic Practice and Action Research*, vol. 28, pp. 87-110, Apr 2015 2015-03-17 2015.

[23] A. C. Bishop and B. R. Cregan, "Patient safety culture: finding meaning in patient experiences," *International Journal of Health Care Quality*

*Assurance*, vol. 28, pp. 595-610, 20152015-07-16 2015.

[24] A. P. Gurses, "Performance obstacles and facilitators, workload, quality of working life, and quality and safety of care among intensive care nurses," 3175496 Ph.D., The University of Wisconsin - Madison, Ann Arbor, 2005.

[25] J. P. Bagian, "Patient Safety: What Is Really at Issue?," *Frontiers of Health Services Management*, vol. 22, pp. 3-16, Fall Fall 2005 2015-04-04 2005.

[26] N. J. Verbakel, M. Langelaan, T. Verheij, C. Wagner, and D. L. Zwart, "Improving patient safety culture in primary care: a systematic review," *J Patient Saf*, 2014.

[27] M. Fleming, "Patient Safety Culture Measurement an Improvement: A "How to Guide"," vol. 8, 2005.

# Survey of Salmonella Contaminated Vannamei Shrimps in Lampung

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## Abstract

The shrimp production in Indonesia increases every year; however, the export of shrimp encounterd problem such as *Salmonella sp* contaminantion. The aim of research was to identify the *Salmonella* contamination on shrimp. The research is done by sample surveys of Vannamei shrimp from the sub district including Wonosobo, Kota Agung, Hanura and RawajituTimur. The survey was done in three replications. *Salmonella* contaminated Vannamei shrimp of aquaculture product in sub district Wonosobo, Kota Agung, and Hanura were taken as samples. The other samples added with natural probiotics such as garlic and noni fruit showed no growth of harmfull bacterium. The average value of organoleptic of vannamei shrimp from sub district Wonosobo, Kotaagung, Hanura and RawajituTimur were above the average 8.07, resulting that it was safely acceptable. So, Organoleptic test not be recommended to microbe analyses.Location of shrimp farming on Padang Cermin (3 x $10^3$colony/g)Kota Agung(1.3 x $10^4$colony/g) and Wonosobo (1.2 x $10^2$colony/g), affected to*Salmonella*sp. contaminanton Shrimp.The good Management of pest and disease in Shrimp farmingon Rawajitudecrease to *Salmonella* sp. content (*Salmonella* sp. = 0). Rawajitu Shrimp farming used a natural antibiotics, such as, garlic and mengkudu/noni fruit. It decrease to *Salmonella* sp. content effectively.

Key words :*Salmonella* sp.,Vannamei shrimp, contaminated, consumer acceptable

## 1. Introduction

The shrimp is a fishery product which became main Indonesia's export commodity. Indonesia's shrimp production is increasing every year. Based on data from the Ministry of Maritime Affairs and Fisheries showed that the shrimp production was increased, in 2009, shrimp production was 338 061 tons, in 2010, increased to 380 971 tons, in 2011, the volume of shrimp production was 400 386 tons (Ministry of Maritime Affairs and Fisheries, 2012), in 2012, was 415 703 tons (Halim, 2013). In 2014, Indonesia's shrimp production was 623,000 tons and in 2015,was785 900 tons (Trobos, 2015). Export to abroad are expected will increase along with the increasing needs of shrimp. In the world, Each shrimp importing countries set their own standards more strict than the standards that was set by the Codex Alimentarius Commission (CAC). For example, Europe Uni countries apply the Rapid Alert System for Food and Feed (RASFF). In Indonesia, The shrimp that will be exported must refer to the standards of government. According to the government standard, the shrimp must not contain Salmonella bacteria, Shigella and Vibrio cholerae (the National Standardization Agency, 2006).

The Shrimp from Indonesia that was contaminated with Salmonella bacteria was rejected by the imported countries. According to the data, the European Union, in 2011, reject 17 of fishery products from Indonesia, and in 2012, the United States rejected 181 of

fishery products from Indonesia that were contaminated with Salmonella (Supriiadi, 2012). The other case, the Food and Drug Administration (FDA) on July 2013 rejected 5 lot vannamei shrimp that were exported from Indonesia, it contain Salmonella contaminant (Maas, 2013). The aim research was to find where the locationn that have *Salmonella* contaminated shrimp. This research was conducted on shrimp taken from the four areas that was listed farming vannamei shrimp in Lampung, such as,the District of Wonosobo, Kota Agung, Hanura and Rawajitu

## 2. Material and Methods

This research was conducted at the Microbiology Laboratory of Food Technology, Lampung University and Pests and Diseases Laboratory, Government Secondary School Kota Agung.Materials that be used are shrimp, ice, alcohol, distilled water, XLD Media, BPWMedia, score sheet. Samples (500g)was taken from the District in Lampung, such, as, Wonosobo, Kota Agung, Hanura and Rawajitu. Sample was carried out by 3 replications. Samples that was taken, be done organoleptic testing and microbial test. Tools that be used was refrigerator, erlenmeyer, test tubes, petri dish, bunsen, measuring cups, glass samples, mask, cotton, paper, rubber, latex gloves, heat-resistant plastic, scales, incubators, laminar flow, needle ose, hot plate.

### Salmonella Test.

Salmonella Testwas done on XLD media (Oxoid). Further cultures are incubated for 24 hours at 37°C. Salmonella colonies had pink with or without black core. Salmonella culture generally havea large coloniesform, core glossy black or almost all colonies look black (SNI 01-2332.2-2006).

## 3. Result and Discussion

### Appearance Tests

Organoleptic testswas done by took samples at shrimp farming location. The shrimp samples was tested by the organoleptic test that needed assessment by the panelists (SNI 01-2728.1 2006). The result research data were presented and displayed on descriptive form, such as, a tables and bar charts forms. The samples was displayedon figures 1. belows.

The average of organoleptic value that was given by panelis on vannamei shrimp from the District of Wonosobo, Kota Agung, Padang Cerminand Eastern Rawajitu (criteria of appearance wasodor and texture)was presented onTable 1 above.

The average organoleptic value of shrimp on appearance, such as, odor and texture criteria of each location was different. The range organoleptic score on each location wasdifferent, with range 8,35 to 9 (Shrimp organoleptic score from Wonosobo was 8.80 to 7.79; Kota Agung8.93 to 7.59:Padang Cermin 8.80 to 8.36. The highest of organolepticscore was the shrimp from Rawajitu with score was 9.

### *1.1.* Salmonella Total

The results of Salmonella Total test showed that some samples was positive *Salmonella*contaminant. *Salmonella*sp. Contaminant was positive detected from Wonosobo, Kota Agung, andPadang Cermin. Salmonella positive sample identification was done by observing the colony grows and Salmonella colonies. The appearance of *Salmonella* colonies on XLD media can be seen on Figure 2.

The test results of *Salmonella*sp. contaminant on post-harvest vannamei shrimp cin Lampungare presented on Table 2 and Table 3 descriptively.

The total *Salmonella* sp test showed that vannamei shrimp on wonosobo, Padang Cermin and Kota Agung district was contamminated by *Salmonella* sp (Table 5).

a. Wonosobo

b. Kota Agung

c. Padang Cermin

d. Rawajitu Timur

Figure1. Vannamei Shrimp Appearance

Table1. Shrimp appearance value that was given by panelis

| **Location** | **Sampel Code** | | | **Average** |
|---|---|---|---|---|
| | **1** | **2** | **3** | |
| Wonosobo | 8,80±0,15 | 7,79±0,62 | 8,54±0,30 | 8,38 |
| Kota Agung | 8,93±0,15 | 8,54±0,30 | 7,59±0,55 | 8,35 |
| Padang Cermin | 8,80±0,15 | 8,80±015 | 8,36±0,28 | 8,56 |
| Rawajitu Timur | 9,00 | 9,00 | 9,00 | 9 |

Location of shrimp farming on Padang Cermin, Kota Agung and Wonosobo affected to *Salmonella* contaminant score. Marine environment is a major source of Salmonella contamination in aquaculture (Olgunoğlu, 2012). Environmental area of Padang Cermin, Kota Agung and Wonosobo was near with ahome society, so that,it supported to *Salmonella* contamination, caused by waste and faecal from waste of home society. Figure farms can be seen on the following figure 3.

According Poeloengan, *et al.* (2009), the source of Salmonella infection in the fishpond was output (excretion) of both animals and humans from animals or humans. *Salmonella*will widespread in the environment, usually, it found in the garbage and materials that be associated with fecal contamination. Padang Cermin cultivation area is also near with the settlement and Fishing Port at Lempasing Beach, Bandar Lampung, so, it increased of Salmonella contamination from the harbor garbage and

household waste. Environmental Kota Agung cultivation area is also relatively near with the residential and Fishing Port Beach Kota Agung. Lack of public awareness to sanitation and hygiene seawater, supported to inreased *Salmonella* contaminant.

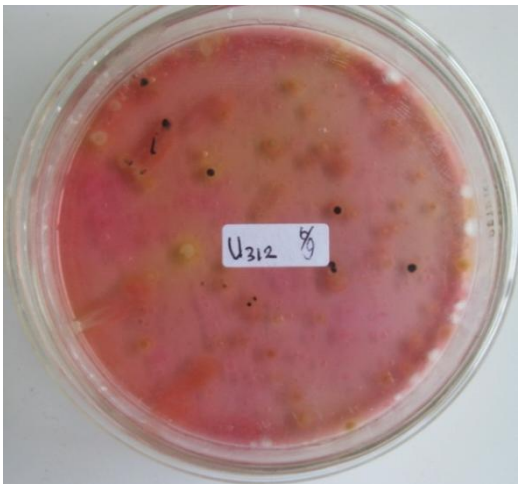



Figure 1. Positive Salmonella on Media XLD

Table 2. *Salmonella* total Test

| Sample Code | Wonosobo | Kota Agung | Padang Cermin | Rawajitu Timur |
|---|---|---|---|---|
| 1 | + | + | + | - |
| 2 | + | - | - | - |
| 3 | + | - | - | - |

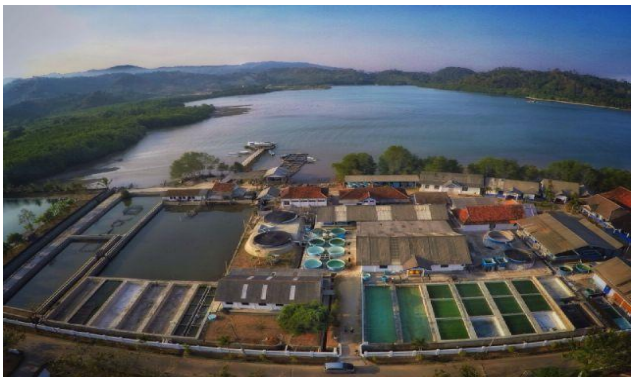
Table 4. Descriptive Data of *Salmonella* contaminanton the District of Wonosobo, Kota Agung, Padang Cermin and Rawajitu

| Area | Sampel | Positive | | Negative | |
|---|---|---|---|---|---|
| | | Total | % | Total | % |
| Wonosobo | 3 | 3 | 100 | 0 | 0 |
| Kota Agung | 3 | 1 | 33,3 | 2 | 66,6 |
| Padang Cermin | 3 | 1 | 33,3 | 2 | 66,6 |
| Rawajitu Timur | 3 | 0 | 0 | 3 | 100 |

Table 5.The quantification of *Salmonella* contaminant on Vannamei shrimps on the district of Wonosobo, Kota Agung, Padang Cermin and Rawajitu

| Sample | Wonosobo | Kota Agung | Padang Cermin | Rawajitu Timur |
|---|---|---|---|---|
| 1 | $1.2 \times 10^2$ | $1.3 \times 10^4$ | $3 \times 10^3$ | - |
| 2 | $1,5 \times 10^5$ | - | - | - |
| 3 | $9.8 \times 10^2$ | - | - | - |

Wonosobo



Padang Cermin

Kota Agung

Rawajitu

Figure 3. Location of Shrimp Farming

Containers of products and the water that was used during handling of shrimp also can be a source of contamination (Prime, 2013). Research Kusumah and Ariesyady (2012) suggested an increase in sanitation and domestic waste treatment will reduce contamination. According to Balfour (2014) Salmonella sp. was affected of temperature control, food handlers, contaminated raw materials, cross-contamination, and transport condition.

According Uddin et al. ((2015) specific sources of Salmonella in aquaculture was the water pond, feed, birds, amphibians, and reptiles. It can lead to fecal contamination.

It was supported by the Muliani, et al (2010) statement,as well as, Widanarni, et al. (2010) that the use of probiotics can reduce the concentration of NH3 that will increase of survival rate of shrimp. The use of probiotics on vannamei shrimp farming can increase the survival rate of shrimp Vannamei (Yudiati, et al., 2010). Application of commercial probiotic vannamei shrimp culture will increase the production of shrimp (Gunarto and Hendrajat, 2002).

During the process of cultivation, the shrimp from East Rawajitu not given chemical drugs. The use of antibiotics in excessive doses can lead to antibiotic-resistant bacteria (Shakir, 2012). Taha, et al (2013) found that*Salmonella* bacteria are resistant to antibiotics, especially the type of co-trimoxazole, 62.2% to gentamicin, ampicillin and 56.6% against 35.5% against nalidixicacid. Pest and Disease Management in Shrimp Fish Rawajitu useda natural antibiotics, such as, garlic and mengkudu/noni fruit. According to Sunanti (2007), secondary metabolites that was contained in garlic are alkaloids, flavonoids, sterols / triterpenoids, essential oils and tannins. The filtrate garlic has inhibitory effect to the growth of *Salmonella* thypimurium.

The use of noni fruit is commonly used to lower blood pressure in humans and noni are known to be antibacterial. The antibacterial properties of shrimp farmers who used to reduce the number of bacteria in farmed shrimp. Bacteria that normally occur in shrimp farming is a bacterium V. harveyi. V. harveyi bacteria cause disease firefly (luminescent vibriosis) on tiger shrimp larvae. Sarida, et al (2010) stated that the noni fruit extract has a real effect in inhibiting the growth of bacteria. This Statement was supported by Efri and Aeny (2004); Puspitasari, et al. (2010); Purwantiningsih, et al (2014);Afrianto and Liviawaty(2011); Sanger(2010); Karnila, et al. (2006); and Harsojo(2008).

## Conclusion

Locationof shrimp farming on Padang Cermin ($3 \times 10^3$ colony/g)Kota Agung($1.3 \times 10^4$colony/g) and Wonosobo ($1.2 \times 10^2$ colony/g), affected toShrimp *Salmonella*sp content.Pest and Disease Management on Rawajitu Shrimp farming (*Salmonella* sp. = 0) was success because it useda natural antibiotics, such as, garlic and mengkudu/noni fruit. Secondary metabolites that was contained in garlic and noni fruit are alkaloids, flavonoids, sterols / triterpenoids, essential oils and tannins. The filtrate garlic and noni fruithave inhibitory effect to the growth of *Salmonella*sp.

## References

Badan Standardisasi Nasional. 2006. Standar Nasional Indonesia 01-2728.1-2006 Udang Segar-Bagian 1: Spesifikasi. Badan Standarisasi Indonesia. Jakarta.

Bahri. 2008. Beberapa aspek keamanan pangan asal ternak di Indonesia.*Pengembangan Inovasi Pertanian 1 (3)*, 2008 : 225-242

Eddy Afrianto dan Evi Liviawaty. 2011. Pengawetan dan Pengolahan Ikan. Kanisius. Yogyakarta.

Efri dan Titik Nur Aeny. 2004. Keefektifan Ekstrak Mengkudu Pada Berbagai Konsentrasi Terhadap Penghambatan Pertumbuhan Bakteri Ralstonia sp. Secara In Vitro. *J Hama dan Penyakit Tumbuhan Tropika* Vol. 4 No. 2: 83-88.

Ervia Yudiati, Zaenal Arifin dan Ita Riniatsih. 2010. Pengaruh Aplikasi Probiotik Terhadap Laju Sintasan dan Pertumbuhan Tokolan Udang Vanamei (*Litopeneus vannamei*), Populasi Bakteri

Vibrio, serta Kandungan Amoniak dan Bahan Organik Media Budidaya. *Ilmu Kelautan September 2010*. vol. 15 (3) 153-158

Galuh Puspitasari, Sri Murwani, Herawati. 2010. Uji Daya Antibakteri Perasan Buah Mengkudu Matang (*Morinda Citrifolia*) Terhadap Bakteri *Methicillin Resistan Staphylococcus Aureus (MRSA)* M.2036.T Secara *In Vitro. Skripsi.* Program Studi Pendidikan Dokter Hewan. Universitas Brawijaya. Malang

Gunarto dan Erfan Andi Hendrajat. 2008. Budidaya Udang Vanamei, *Litopenaeus vannamei* Pola Semi Intensif dengan Aplikasi Beberapa Jenis Probiotik Komersial. *Jurnal Riset Akuakultur* Vol. 3 No. 3 Tahun 2008: 339-349

Halim. 2013. Petambak Dan Perdagangan Bebas Komoditas Udang. Diunduh dari http://www.kiara.or.id/petambak-dan-perdagangan-bebas-komoditas-udang/ pada 08-06-2014 20:18

İlkan Ali Olgunoğlu. 2012. *Salmonella* in Fish and Fishery Products, *Salmonella* - A Dangerous Foodborne Pathogen, Dr. Barakat S M Mahmoud (Ed.), ISBN: 978-953-307-782-6, InTech. Diunduh dari : http://www.intechopen.com/books/salmonella-a-dangerous-foodborne-pathogen/salmonella-in-fish-andfishery-products pada 23 Januari 2014 15:03

Kementerian Kelautan dan Perikanan. 2012. Kelautan dan Perikanan Dalam Angka 2011. Kementerian Kelautan dan Perikanan. Jakarta

Maas. 2013. Wholey Brand Cooked Shrimp Recall Issued Due To *Salmonella* Risk. Lawsuits.Com. Diunduh dari Http://www.Aboutlawsuits.Com/Shrimp-Recall-Salmonella-Risk-51148/ pada 21 Nopember 2013 14:52

Masniari Poeloengan, Iyep Komala dan Susan M. Noor. 2005. Bahaya *Salmonella* Terhadap Kesehatan. *Prosiding Lokakarya Nasional Penyakit Zoonosis*. Bogor. p216-223

Muliani, Nurbaya, dan Mujiharijadi Atmomarsono. 2010. Penggunaan Probiotik Pada Pemeliharaan Udang Windu *(Penaeus monodon)* dengan Dosis yang Berbeda. *Prosiding Forum Teknologi Akuakultur 2010.* 249-259

Munti Sarida, Tarsim dan Iwan Faizal. 2010. Pengaruh Ekstrak Buah Mengkudu (*Morinda citrifolia L.*) dalam Menghambat Pertumbuhan Bakteri *Vibrio harveyi* Secara *In vitro*. *Jurnal Penelitian Sains* 2010 Volume 13 Nomer 3(D) 13312.

Sunanti. 2007. Aktivitas Antibakteri Ekstrak Tunggal Bawang Putih (*Allium sativum* Linn.) Dan Rimpang Kunyit (*Curcuma domestica* Val.) Terhadap *Salmonella typhimurium*. Skripsi. IPB. Bogor.

Supriadi. 2012. Mengandung Salmonela, Produk RI Sempat Ditolak AS.www.surabayapost.co.idRabu, 03/10/2012 | 12:14 WIB Diunduh dari http://www.seafoodservicecentre.com/index.php?option=com_content&view=article&id=201%3Amengandung-salmonela-produk-ri-sempat-ditolak-as-&catid=34&Itemid=1

Taufik H, Endang S.W dan Setyawati S.K. 2013. Pengaruh Ekstrak Buah Mengkudu *(Morinda citrifolia)* Terhadap Aorta Terpisah Marmut *(Cavia porcellus)* Tanpa Endotel. *Jurnal Kedokteran Brawijaya.* Vol 19. No. 3

Theresia Ika Purwantiningsih, Yustina Yuni Suranindyah dan Widodo. 2014. Aktivitas Senyawa Fenol Dalam Buah Mengkudu (*Morinda Citrifolia*) Sebagai Antibakteri Alami Untuk Penghambatan Bakteri Penyebab Mastitis. *Buletin Peternakan Vol. 38(1): 59-64*, Februari 2014

Trobos. 2015. Perkuat Dominasi Udang Indonesia. Diunduh dari http://www.trobos.com/detail_berita.php?sir=12&sid=5856 pada Senin, 20 April 2015

Wibowo, Muliana, Prabowo. 2010. Analisis Residu Antibiotik Kloramfenikol Dalam Daging Ikan Gurami *(Osphronemus gouramy, Lac)* Menggunakan Metode High Performance Liquid Chromatography. *Jurnal Ilmiah Farmasi* Volume 7 Nomor 1 Tahun 2010

Widanarni, M.A. Lidaenni, D. Wahjuningrum. 2010. Pengaruh Pemberian Bakteri Probiotik *Vibrio* SKT-b dengan Dosis Yang Berbeda Terhadap Kelangsungan Hidup Dan Pertumbuhan Larva Udang Windu (*Penaeus monodon*) Fab. *Jurnal Akuakultur Indonesia* 9 (1), 21–29 (2010)

# Determinants of Birth Weight at Various Quantiles in West Sumatra

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## Abstract

Covariates could affect the responses differently at various points of the response distribution. Many covariates might have higher impact on conditional mean of the response than on conditional 10th percentile, for example. These effect can be analyzed directly by using quantile regression. This paper aims to implement the use of quantile regression to identify the determinants of birth weight at various quantiles. A cross-sectional study was conducted in March to June 2016 by distributing questonnaires to mothers who gave birth at any selected hospital in West Sumatra. This research proves that determinants of birth weight at low quantile are education level, problems during pregnancy and prenatal care. Meanwhile parity, pragnancy spacing, problems during pregnancy and gender are associated with higher birth weights. All proposed model could be accepted based on goodness of fit test.

Keywords: quantile regression, cross-sectional study, birth weight

## 1. Introduction

Birth weight has served as leading indicator of infant health, with low birth weight infants classified as those weighing less than 2500 grams at birth. Obsevable measures of mother weight gain, education level, problems while pregnant, age, parity, prenatal care, mother weight gain, hemoglobin (Hb) and spacing pregnancies (Abrevaya& Dahl, 2008; Burgette & Reiter, 2012) were assummed had strong associations with birth weight. For instance, according to a report by Burgette & Reiter (2012), mothers who had problems during pregnancy would have babies with birth weight less than 2500 grams.

Many researches have examined that the low birth weight will cause many problems. Abrevaya in his article wrote that the infant mortality rate increases at lower birth weights. The direct medical costs for babies with low birth weight are quite high as well. The babies with low birth weight have the long term effects on their cognitive development, educational outcomes and labor market outcomes. The babies would have development problems in cognition, attention and neuromotor functioning that persist until adolescence (Hack et. Al., 1995). The babies with low birth weight are more likely to attend special class, delay entry into kindergarden or repeat a grade in school(Corman and Chaikind, 1998). Those babiesare also more likely to have inferior labor-market outcomes, being more likely to be unemployed andearn lower wages (Behrman and Rosenzweig (2004); Case et. al. (2005); Currie and Hyson (1999)).

Although it has received less attention in the economics literature, highbirthweight outcomes can also represent adverse outcomes. For instance, babies weighing more than 4000 grams, classified as high birthweight (HBW) and especially those weighing more than 4500 grams, classified as very high birthweight (VHBW) are more likely to require cesarean-section births, havehigher infant mortality rates, and develop

health problems later in their life.

A difficulty in evaluating initiatives aimed at improving birth outcomes is to accurately estimate the causal effects of prenatal activities on these birth outcomes. Unobserved heterogeneity among childbearing women makes it difficult to isolate causal effects of various determinants of birth outcomes. Whether or not a mother's age affect the infant weight, for instance, is likely to be correlated with unobserved characteristics of the mother. To deal with this difficulty, various studies have used an instrumental-variable approach to estimate the effects of prenatal care (Currie and Gruber (1996); Evans and Lien (2005); oyce (1999)), and air pollution (Chay and Greenstone (2003a, 2003b)) on birth outcomes.

Another approach has been to utilize panel data (i.e., several births for each mother) to identify these effects from changes in prenatal behavior or maternal characteristics between pregnancies (Abrevaya (2006); Currie and Moretti (2002); Rosenzweig and Wolpin (1991); Royer (2004)). One concern with the panel-data identification strategy is the presence of "feedback effects," specifically that prenatal care and mother's habbits in later pregnancies may be correlated with birth outcomes in earlier pregnancies. Royer (2004) provides an explicit estimation strategy to deal with such feedback effects (using data on at least three births per mother). Since the costs associated with birthweight have been found to exist primarily at the low end of the birthweight distribution (with costs increasing significantly at the very low end), any studies have estimated the effects of birth inputs on the fraction of births below various intervals. This present study conside a quantile regression approach to estimate the effects of birth inputs on birthweight.Quantile approach provides a method for determining how birth inputs affect birthweight at different parts of the distribution. The birth inputs involved in this study are 8 indicators consists of continuous and categorical types, they are education level, problems while pregnant, age, parity, prenatal care, mother weight gain, hemoglobin (Hb) and spacing pregnancies (Abrevaya& Dahl, 2008; Burgette & Reiter, 2012).

There are any advantages using quantiles regression then analysis of variance or classical regression. Any reasons why we better use quantile regression are :

- Analysis of variance (ANOVA) and regression provide information only about the conditional mean.
- More knowledge about the distribution of the statistic may be important.
- The distribution of , the dependent variable,conditional on covariate X, may have thick tails.
- The conditional distribution of Y may be asymmetric.
- The conditional distribution of Y may not be unimodal.
- Neither regression nor ANOVA (analysis of variance) will give the robustresults, especially if theoutlier existinside the data.

## 2. Material and Methods

In this present study, we used primary data collected by distributing the questionnaires to mother who just have baby, live, single and stay in West Sumatra. The questionnaires were distributed from March to July 2016. There are 93 respondents with complete information that involved in this study.

The response variable is birth weight, recorded in kilograms. Meanwhile the birth inputs are assummed affeced by eight indicators consists of continuous and categorical types. There are education level, problems while pregnant, age, parity, prenatal care, mother weight gain, hemoglobin (Hb) and spacing pregnancies.The following Table 1 presents the summary statistics of Birthweight data.

Table 1. Descriptive of Birth Weight Data

| | | |
|---|---|---|
| Mean | | 3,063 |
| Median | | 3,100 |
| Mode | | 3,2 |
| Skewness | | -0,592 |
| Kurtosis | | 0,578 |
| Minimum | | 1,1 |
| Maximum | | 4,5 |
| Percentiles | 25 | 2,700 |
| | 50 | 3,100 |
| | 75 | 3,500 |

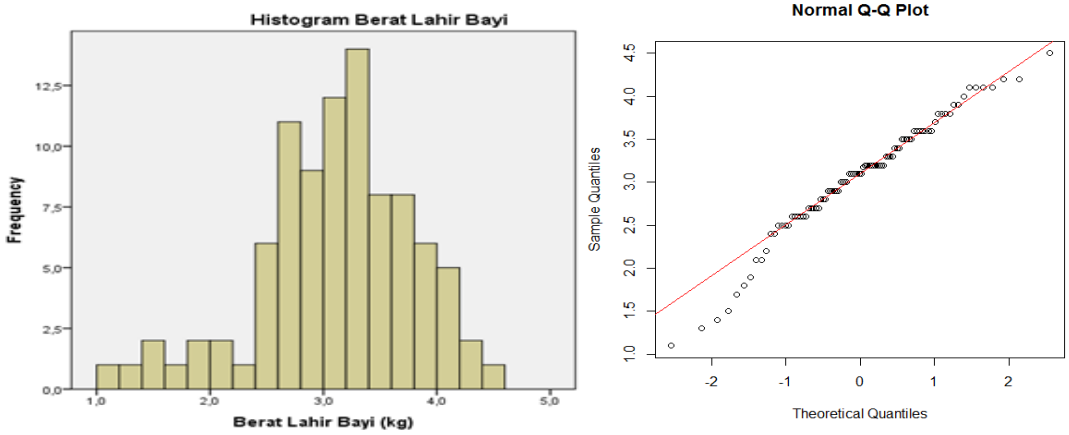
Based on the description in Table 1 we are informed that the mean of birthweigth data is 3,063. The highest value of the data is 4,5 and the lowest value is 1,1. We also see that the distribution of the data is skewed to the left since its skewness is - 0,592, as presented in Figure 1.(a).

For construction of Birthweight model, this present study apply the quantile regression approch, since we purpose to identify the Birthweigth model for any quantiles (low quantile, middle quantile and high quantile). The following is the general explanation regard quantile procedure used in this research.

As described by Gilchrist (2000), quantile is defined as the value that corresponds to a specified proportion of a sample or population. Thus, we may defined $\tau$ thquantile as the value which divide the data into two parts, the $\tau$ fraction of the data below it and 1-$\tau$ fraction of the data above it, and 0< $\tau$ <1. Median is a very commonly used quantile, which is aqual to a proportion of 0.5 is the ordered data. Regression analysis is used to quantify the relationship between a responce variable and one or several of free covariates (Yanuar, 2013).

Quantiles regression as an introduced by Koenker and Basset (1978), is a statistical method used to estimate models for conditional quantile functions. Unlike the classical linear regression methods that are based on minimizing sums of squared residuals and to estimate models for conditional mean functions, quantile regression methods are based on minimizing absolute residuals, and intended to estimate conditional median functions and a full range of other conditional quantile functions. Quantile regression also provides a more complete graph of the conditional distribution of variable ot interest $Y$ given $X = x$.

For a random sample $\{y_1, \ldots, y_n\}$, the classical linear regression can be estimated by the well-known method, which minimizes the sum of squared residuals:




(a) (b)

Figure 1.(a) Histogram of Birthweight data.
(b) Empirical quantile plot and Normal plot of Birthweight data.

$$\min \sum_{i=1}^{n} (y_i - \hat{y}_i)^2 \quad (1)$$

For the special case of estimation the conditional median of function, we can define the solution as problem to minimization a sum of absolute residuals, where there are the same numbers of observations above and below the median, which can be calculated by:

$$\min \sum_{i=1}^{n} |y_i - \hat{y}_i| \quad (2)$$

Analogous to the concept of median, Koenker and Basset (1978) proposed a complete and different method for estimation of an unknown value, say a, for any $\tau$ in the interval (0,1), which may be defined as any solution to the minimization problem of the equation:

$$\min_{a \in \Re} \left\{ \sum_{i=1}^{n} \tau \, |y_i - a| + \sum_{i=1}^{n} (1-\tau) \, |y_i - a| \right\} \quad 0 < \tau < 1 \quad (3)$$

Consider a classical linear regression model $y_i = \boldsymbol{x}_i' \boldsymbol{\beta} + e_i$, we defined a linear model for the $\tau$ -thquantile as:

$$y_i = \boldsymbol{x}_i' \boldsymbol{\beta}_\tau + e_i \quad i = 1, \ldots, n \quad (4)$$

In estimating models for conditional quantile function, we minimize a sum of asymmetrically weighted absolute residuals. This will contribute to different weights to positive and negative residuals. The general $\tau$ -th sample quantile, which is the analogue to equation (3) can be formulated as:

$$\hat{\beta}(\tau) = \min_{\beta \in \Re} \left\{ \sum_{i=1}^{n} \tau \, |y_i - \boldsymbol{x}_i' \boldsymbol{\beta}_\tau| + \sum_{i=1}^{n} (1-\tau) \, |y_i - \boldsymbol{x}_i' \boldsymbol{\beta}_\tau| \right\}$$

And equivalently written as:

$$\hat{\beta}(\tau) = \min_{\beta \in \Re} \sum_{i=1}^{n} \rho_\tau \left( y_i - \boldsymbol{x}_i' \boldsymbol{\beta}_\tau \right) \quad 0 < \tau < 1 \quad (5)$$

Several software packages can be used to implement the quantile regression method, such as S-plus, R-program and Stata. In this research, R software was used to analyze data of the Birthweight.

## 3. Result and Discussion

In this analysis, quantile regression approach is used to examine the relationships between the Birthweight and some potential explanatory variables. Table 2 provides a summary of describe the explanatory variables which are found to have a significant relationships with Birthweight for various conditional quantile function, particularly for τ equals 0.10, 0.25, 0.50, 0.75 and 0.90. In the last column are the estimated of ordinary least square approach and its corresponding standard errors in the brackets.

Table 2 informs us that mother with middle or high education level tent to have havier baby than mother with low education level. Mother with more parities tent to have havier baby than less parities. Mother with longer space of pregnancies tent to have ..... baby. The heavier of mother's weight gain the heavier of birthweight of her baby. The higher of mother's hemoglobin, the heavier of her baby's birthweight. Thus this study found that significant variables which effect the baby's birthweight are education level, parity, spacing pregnancies, mother's weight gain and mother's hemoglobin.

Table 3 presents the goodness of fit for all model, indicated by *PseudoR*$^2$ value. Based on the result of this study, it proved that all model at any selected quantiles are acceptable since all *PseudoR*$^2$ values more than 0.7. The best model is at middle quantile (*PseudoR*$^2$equals 0.910).

Table 2. Coefficient Estimated for Birth Weight Model Using Quantile Regression (QR) and OLS

| Indicator Variable | Estimate of QR (Standard Error) | | | | | Estimate of OLS (Standard Error) |
|---|---|---|---|---|---|---|
| | τ = 0.10 | τ = 0.25 | τ = 0.50 | τ = 0.75 | τ = 0.90 | |
| $\beta_2$(Middle) | 0.700 (0.224)** | 0.501 (0.243)** | 0.378 (0.173)** | 0.421 (0.262) | 0.203 (0.228) | 0.364 (0.174)** |
| $\beta_3$ (High) | 0.662 (0.345)** | 0.433 (0.258)* | 0.378 (0.184)** | 0.613 (0.279)** | 0.149 (0.243) | 0.420 (0.178)** |
| $\beta_4$ (Parity) | 0.331 (0.137)** | 0.235 (0.102)** | 0.106 (0.073) | 0.216 (0.111)** | 0.279 (0.096)** | 0.242 (0.073)** |
| $\beta_5$ (Spacing pregnancies) | -0.089 (0.057) | -0.070 (0.042)* | 0.014 (0.030) | -0.039 (0.046) | -0.074 (0.040) | -0.052 (0.030)* |
| $\beta_6$ (Weight gain) | 0.038 (0.025) | 0.023 (0.019) | 0.033 (0.013)** | 0.006 (0.020) | 0.015 (0.017) | 0.021 (0.013) |
| $\beta_9$ (Hb) | 0.108 (0.099) | 0.130 (0.074)* | 0.097 (0.052)* | 0.062 (0.080) | 0.126 (0.069)* | 0.077 (0.053) |

* Significant at 10% level
**Significant at 5% level

Table 3. *PseudoR*$^2$ for Selected Quantile for Low Birth weight Cases

| Quantiles | *PseudoR*$^2$ |
|---|---|
| 0.10 | 0.736 |
| 0.25 | 0.877 |
| 0.50 | 0.910 |
| 0.75 | 0.909 |
| 0.90 | 0.856 |

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## References

[1] Abrevaya J. and Dahl MC. 2008. The Effect of Birth Inputs on Birth weight: Evidence from Quantile Estimation on Panel Data. *Journal of Business & Economic Statistics*, **26**, 379 –397

[2] Burgette LF. and Reiter JP. 2012. Modeling Adverse Birth Outcomes via Confirmtory Factor Quantile Regression. *Biometrics* **68**, 92 –100

[3] Davino C, Furno M, and Vistocco D. 2014. *Quantile Regression: Theory and Applications*. John Wiley & Sons, Ltd.

[4] Feng X and Zhu L. 2016. Estimation and Testing of Varying Coefficients in Quantile Regression. *Journal of the American Statistical Association***111**, 266 – 274.

[5] Feng, X., He, X., and Hu, J. 2011. Wild Boostrap for Quantile Regression. B*iometrica*, 98, 995–999.

[6] He X. and Zhu LX. 2011. A lack of fit test for quantile regression. *Journal of the American Statistical Association*, 98 (464) : 1013 - 1022

[7] Hulman A., et. Al. 2015. Heterogeneous Effect Of Gestational Weight Gain On Birth Weight: Quantile Regression Analysis From A Population-Based Screening. *Annals Of Epidemiology*, 25: 133 – 137

[8] Wang, H., Zhu, Z., and Zhou, J. 2009. Quantile Regression in Partially Linear varying Coefficient Models. *The Annals of Statistics*, 37, 3841–3866.

[9] Wei Y and Carroll RJ. 2008. Quantile Regression With Measurement Error. *Journal of the American Statistical Association*, 104: 1129 - 1143

[10] Wei, L., Wang, D. and Hutson, AD. 2015. An investigation of quantile function estimators relative to quantile confidence interval coverage. *Communication Statistics Theory Methods*, 44 (10): 2107 – 2135.

[11] Yanuar F. 2014. The Estimation Process in Bayesian Structural Equation Modeling Approach. *Journal of Physics : Conference Series*, **495,** 012047.

# Detection Of Osteoporosis in Ovariohysterectomized Cats (*Felis Domesticus*) based on Serum Osteocalcin Levels

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## Abstract

The purpose of this study was to investigate the changes of bone density and the possibility of osteoporosis based on serum osteocalcin levels in ovariohysterectomized cats. Twenty cats at the age of 5-8 years oldwere used in this study, consisted ten cats that have been ovariohysterectomized for at least six months earlier and ten intact cats. Cats that met the selection criteria were collected from their owner. The selection criteria were healthy, not consuming any drugs contains substances that influence bone health and bone mass. Furthermore, all cats have blood drawn through a vein r*adialischepalicaantibranchii anterior* as much as about 2 ml. Statistical analysis of *Independent Samplet-test* was used to compare the levels of osteocalcin between the two groups of samples with a confidence level of 95%. The result of this study did not show any significant differences in osteocalcin levels comparison between ovariohysterectomized cats and intact cats ($p>0.05$). In conclusion, it could be stated that thecats at the age of 5-8 years old and it had been ovariohysterectomized for at least six months earlier, that had not shown significant differences in osteocalcin levels compared with intact cats, despite the fact that there was a tendency towards osteocalcin levels increased sixfold in ovariohysterectomized cats.

Keywords: osteocalcin; osteoporosis, ovariohysterectomized, bone density, domestic cats

## 1. Introduction

Ovariohysterectomy is one type of surgery that often carried out in the veterinary practice. Ovariohysterectomy in cats and dogs usually done with the purpose of sterilization to limit the number of population, and also as a medical procedure because of reproductive disorders. There are few long-term side effects as a result of ovariohysterectomy such asmusculoskeletal disorders, including decreased levels of bone density [1].

A decrease in bone density is a sign of osteoporosis. Osteoporosis is a metabolic bone disease characterized by adecrease in bone mass, due to reduced bone mineral matrix and accompanied by destruction of micro-architecture bone tissue, with aconsequent decrease in bone strength, resulting in a tendency of bones fracture[2]. The decrease in bone mass and the deterioration of bone tissue's architecture, closely related to the estrogen deficiency due to ovariohysterectomysurgery.

Estrogen is a steroid sex hormone that holds avery important role in bone metabolism, affecting the activity of osteoblasts and osteoclasts, include maintaining the work balance of both cells by regulating the production of paracrine factors primarily by osteoblasts. In animal studies, estrogen deficiency leads to osteoclastogenesis and bone loss occurred. Giving estrogencauses the bone reformation, and found decreased production of IL-1, IL-6, and TNF-α, which are cytokinesthat functioned in bone resorption. Estrogen also

stimulates the expression of osteoprotegerin (OPG) and TGF-β (Transforming Growth Factor-β) in osteoblasts and stromal cells, which further inhibits bone resorption and increase the evidence of osteoclast cells apoptosis[3],[4].

Early detection of osteoporosis is very important in order to prevent further progression of the disease. The last few years has been known to examined of thebone marker as osteocalcin, β-CrossLaps, P1NP and alkaline phosphatase (ALP). In contrast to the examination of Bone Mineral Density (BMD), the purposes of bone marker analysisare monitor and assess response to treatment, the diagnosis of patients with risk of osteoporosis, looking for the cause of bone reduction rapidly, choosing the appropriate treatments, monitoring patients with corticosteroid treatment and study the pathogenesis of osteoporosis [5].

The bone marker can give a sign of bone remodeling process that is going on. This examination covers a bone resorption marker performed by osteoclasts and bone formation marker conducted by osteoblasts. Based on these conditions, many researchers prefer the analysis of bone marker for managing osteoporosis [6].

Osteocalcin is highest non-collagen protein in bone and its produced by osteoblasts, the cells that involved in bone formation. Osteocalcin is a protein that depends on thepresence of vitamin K and vitamin D. Osteocalcin fragments also released into the bloodstream and can be measured in levels. There is aform of intact osteocalcin and NMID-fragment in the bloodstream. Therefore, examination of osteocalcin level is a good parameter for determining bone metabolism disorders in terms of bone formation and bone turnover and can be used to predict the speed of decline in bone mass density and treatment succeed[7],[8],[9].

Study on the incidence of osteoporosis in ovariohysterectomized cats has not been widely reported. A studyreported that there were no significant differences in bone density and lumbar bone mineral composition in puppies aged 10 weeks old which not ovari ohy sterectomized with ovariohy sterectomized puppies for 6 months [10]. Another research also reported that there is no risk of osteoporosis until 6 months post ovariohysterectomy in kittens aged 3 months old [11]. Previous studies emphasize the risk of the incidence of osteoporosis should be at 6 months post ovariohy sterectomy. So it is necessary to study the detection of osteoporosis in post-ovariohysterectomy cats over 6 months and emphasized in adult cats that have been grown by examination of blood serum osteocalcin levels to predict the pace of decline in bone mass density.

The outcomes of this study are expected to provide information about the effect to pet owners who will proceed ovariohysterectomyto their beloved pets, so it can manage early preventative planning andwould not develop further osteoporosis.

## 2. Material and Methods

This research is an analytic observational study with cross sectional approach. The study conducted at the Veterinary Teaching Hospital Faculty of Veterinary Medicine Universitas Airlangga for data collection and blood samples in cats.

Based on the results of sample number counts used in this study, therewas twenty adult local cats which are further divided into 2 groups of 10 cats that had been ovariohysterectomized (OH) and 10 cats that had not been ovariohysterectomized (NOH). OH, groups that were used in this study are cats that had been ovariohysterectomy at 6 least months minimum time period.

The sampling technique was accidental sampling. Based on the database of patients in Veterinary Teaching Hospital Universitas Airlangga and from aprivate veterinary practitioner, data taken from adult female cat aged 6-8 years. The next step of this

studycame to patients address that met inclusion criteria which are healthy, not currently took any medications that can affect bone health, subsequent interviews and took informed consent, signed a consent as respondents in participating in this study.All of the procedures was done after conducted anamnesis and physical examination. Furthermore, approximately 2 ml blood sampling performed in all groups of cats through the radial vein anterior chepalicaantibranchii for examination of serum osteocalcin. Osteocalcin serum examination was using the Elecsys N-MID Osteocalcin Assay with the sandwich method of two monoclonal antibodies specific to a significant group of antigen (epitope) in N-MIDfragment and N-terminal fragment, results in units of ng / mL [12],[6],[13].

The data was obtained with data normality test with the Kolmogorov-Smirnov testprior to the first analysis of the differences. If the data obtained anormal distribution, the difference test conducted with two different mean test with Independent Sample t-test. If the distribution of the data is not normal, it proceeds with the Mann-Whitney test. Moreover, to determine whether there is a relationship between independent variables and dependent variables along with statistical significance relation with chi-square test by looking for value OR (Odds Ratio). Data analysis using SPSS for Windows version 16.

## 3. Result andDiscussion

### Serum Osteocalcin Levels of OH and NOH Cats

Based on a statistical test of normality, serum levels of osteocalcin data shows a normal distribution, so as the difference test of data can be done by Independent Sample t-test. The result of the analysis of serum osteocalcin levels in the group of ovariohysterectomized cats (OH) and ovariohysterectomized cats (NOH) is presented in table 1.

Table 1. Serum Osteocalcin Levels of OH and NOH Cats

| Group | Normality Test Kolmogorov-Smirnov | Osteocalcin Levels (ng/ml) |
|---|---|---|
| OH | 0,385/p=0,998 | 6,08 ± 1,94[a] |
| NOH | 0,917/p=0,37 | 4,51 ± 1,72[a] |

Description :

- Normality test (p>0,05), the data distributed normally
- a superscript that put in the same column showed no significant difference (p>0,05)

Based on these data, it revealed that the levels of osteocalcin in cats OH group with NOH has no significant differences (p> 0.05). However, it considered there was slightlyincreaseof osteocalcin levels in cats OH group. This may be due to the age of cats which used as a sample in this study are still fairly young to have any signs of significant bone loss.Another possibility that might influence theresult of the osteocalcin levelswas the time between the surgery of ovariohysterectomy up to examination osteocalcin levels that average 1-2 years are used as samples in this research can also affect the appearance of bone loss level. On the amount of short period of time, there is a possibility that estrogen levels in the body have not been completely lost because of the homeostasis effects, so that the progressdeclined gradually. This causes the bone destruction process by osteoclasts are not very big because it stillhas effects of estrogen in the blood which are able to stimulate osteoblast activity and bone differentiation, with the osteocalcin expression.

In this study, the levels of osteocalcin in the group of cats in OH looks higher than in thegroup of cats in NOH. Osteocalcin is a marker of bone formation which is a non-collagen protein in bone matrix that biosynthesized by osteoblasts during the active process of bone repair as their reaction of osteoclast activity because of the influence of estrogen levels due to the removal of the ovaries.

The absence of differences in osteocalcin level between OH cat group with NOH cat group does not mean that cats in OH group did not have the risk of increased osteocalcin levels which is a good parameter for determining disturbances of bone metabolism in bone formation and turnover of bone.It can be used to predict the speed of decline in bone mass density and treatment success. Based on this calculation, it is necessary to calculate the risk factors of increased osteocalcin levels in cats that have been in ovariohysterectomized.

Based on the calculation of odds ratios (Table 2) that the estimated risk of an increase of osteocalcin levels in cats group OH is 6 times higher than the cat'sgroup NOH.The value of common odds ratio indicates that the cat is in OH have a greater riskof a0,81-fold increase in the occurrence of osteocalcin levels, and the biggest risk is 44.351 times an increase in osteocalcin levels. Asymp value Sig (2-sided) in this study was> 0.05 then the level of 95% declared insignificant that it cannot represent the entire population, which means there are many other factors affecting the increase in serum osteocalcin levels.

Table 2. Odds ratios OH CatsGroup to Increased Levels of Osteocalcin

| Analysis | OH Cats Group |
|---|---|
| Odds ratio (95%) | 6 |
| p value | 0,79 |
| Common odds ratio | Min : 0,8 |
| | Max : 44,351 |

The same opinion was stated by a study, declares that the osteocalcin is a marker of bone formation, and in some situations such as corticosteroid therapy can be more sensitive than serum alkaline phosphatase activity. Osteocalcin serum reflects approximately 10-40% of the production of osteocalcin which is not related to the bone matrix. Osteocalcin fragment may be derived from bone resorption and catabolism of in vivo molecules prior to clearance that occurred by metalloprotease in the kidneys and liver [13].

Another study also reported that increased biochemical markers of bone formation like osteocalcin levels, the higher the risk of fractures due to osteoporosis. It turned out to have different variability, and there is no accurate data on how the use of these markers when compared with the examination of bone mineral density (BMD) in osteoporosis [14].

## Conclusion

Based on the results of this study, it can be concluded that the levels of osteocalcin on the local cat aged 6-8 years old who have been ovariohysterectomizedat least 1 year, showed no significant difference with the aged 6-8 years old non-ovariohysterectomized local cats. However, the riskestimationin increased osteocalcin levels in OH cats group is 6 times higher than NOH cats group, though the extent of confidence level 95% did not showsignificantly.

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## References

[33] I. M.Reichler, "Gonadectomy in cats and dogs: Review of risk and benefits," *Reprod Domest Anim.*, vol. 44, issue s2, pp. 29–35, July. 2009.

[1] G. R. Mundy, *Bone Remodeling And Its Disorders*,Philadelphia, USA: Martin Dunitz Ltd., 1995. p. 172-207

[2] N. H.Bell, "RANK ligand and the regulation of skeletal remodeling," *J Clin Invest.*, vol. 111, issue 8, pp. 1120–1122, April. 2003.

[3] D. H. Jones, Y. Y. Kong, and J. M. Penninger, "Role of RANKL and RANK in bone loss and

arthritis," *Ann Rheum Dis.*, vol. 61,suppl2, pp. ii32–ii39, Nov. 2002.

[4] M. Suryaatmadja, "Peran pertanda tulang pada pengelolaan osteoporosis," in *Simposium Pendidikan Berkesinambungan Patologin Klinik*, 2005, p. 29-41.

[5] L.Chailurkit, B. Ongphiphadhanakul, N.Piaseu, S. Saetung, and R. Rajatanavin, "Biochemical markersof bone turnover and response of bone mineraldensity to intervention in early postmenopausalwomen: an experience in clinical laboratory," *Clin Chem.*, vol. 47, issue 6, pp. 1083–1088, June. 2001.

[6] M. Kaniawati, and F. Moeliandari, "Penanda biokimia untuk osteoporosis," in *Forum Diagnosticum Prodia Diagnostics Educational Services*, 2003, paper 1, p. 1-18.

[7] R. S.Filip, and J. Zagórski, "Age-and BMD-related differences in biochemical markers of bone metabolism in rural and urban women from Lublin Region, Poland," *Ann Agric Environ Med.*, vol. 11, issue 2, pp. 255–259, Jan. 2004.

[8] H. Isbagio, in "Panduan Diagnosis dan Pengelolaan Osteoporosis," *Pengurus Besar Ikatan Rematologi Indonesia (IRA)*, 2005, p. 1-31.

[9] H. Ekici, B. H. Sontas, T. S. Toydemir, Ö. Senmevsim, L. Kabasakal, and Y. Imre, "The effect of prepubertal ovariohysterectomy on spine 1 mineral density and mineral content in puppies: apreliminarystudy," *Res Vet Sci.*, vol. 82, issue 1, pp. 105–109, Feb. 2007.

[10] M. Uçmak, Ö. T.Yılmaz, M. C. Gündüz, Z. G. Uçmak, O.Duzgun, N. Eskiyurt, C. U. Oruç, S. Genç, Ö. M. Erzengin, and E. Karaçam, "Osteoporotic risk and physeal closure in prepubertal ovariohysterectomized cats," *Anim Reprod Sci.*, vol. 161, pp. 146–151, Oct. 2015.

[11] J. L.Allison, S. Hodges, and R. Eastell, "Measurement of osteocalcin: Review article," *Ann Clin Biochem.*, vol. 37, issue 4, pp. 432–446, Jul. 2000.

[12] K. Ivaska, "Osteocalcin: Novel insights into the use of osteocalcin as a determinant of bone metabolism," Thesis, Department of Anatomy, Institute of Biomedicine and The National Graduate School for Musculoskeletal Diseases (TULES), University of Turku, Turku, Finland, 2005.

[13] P.Garnero, and P. D. Delmas, "Contribution of bone mineral density and bone turnover markers to the estimation of risk of osteoporotic fracture in postmenopausal women," *J Musculoskel Neuron Interact.*, vol. 4, issue 1, pp. 50–63, Mar. 2004.

# Influence of Education and Local Wisdom on Environment Villages in Minangkabau

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## Abstract

The aim of research was to uncover the effect of local wisdom to the development of environment Nagari in Minangkabau. The object of research is NagariParingan, Nagari Sungai Antuan, Nagari Barung Barung Balantai Selatan, NagariSikucur and NagariParu. The research method is quantitative. The results of linear are 1). The influence of the level of education to the development of environment Nagari = 0.107, 2). The influence of local wisdom to the development of environment Nagari = 0.447, 3). The results are multiple influences of education level and local wisdom together towards environmental development Nagari = 0.478. Local knowledge can be assessed from behavior, practices and ethics of the community in managing the environment wisely. He advised the public to be more aware of the importance of environmental development towards better assisted public and private parties.

Keywords: education, local wisdom, development environment in Minangkabau

## 1. Introduction

Declining quality of the environment caused by human activities that do excessive exploitation of natural resources. Using natural resources without regard to the capacity of the environment and ecological functions can damage the environment. According Suranto and Kusrahmadi (1993) stated that the damage to the environment due to human activities are mainly due to: 1) the ignorance of the public against the result of his actions, such a habit of throwing garbage in the river or anywhere that is not realized will cause pollution, 2) urgency life, so unwittingly activities damage the environment continues as mining wood for burning brick has become a job and family income, 3) lack of knowledge about the balance and function of ecosystems, such as the use of pesticides that unwittingly lead to the extinction of other organisms, 4) concern the low environmental sustainability for example industrial disposal of waste without considering its impact on the environment, 5) less understand environmental laws and lack of legal sanction for violators.

Cultural life of harmony invitation nature have been taught for generations in the life of society. But the swift currents of globalization that resulted in community consumptive lifestyle. Culture loving environment is now thinned, as people who have a caring attitude and behavior towards the environment has also been reduced. Attitude and concern for the environment starts from self-awareness as part of the ecosystem, so that the ecosystem can be maintained. That awareness is embedded in the culture of the community in the form of local wisdom.

The results of observations dated August 20, 2016, in the District Fifty City, District Mungka most local water supplies originate from the hills that surround Nagari itself, but the fact is now on long droughts causing drought well water for their daily needs and water to the rice fields. This happens because of a lack of community

cohesiveness maintain existing water source in the hills. Allegedly this is the case because it has reduced the number of trees or vegetation in the forest plant disturbed, so water is usually always there, now people lack clean water sources. It also occurs in Sijunjung, shows that people can not continue cultivating rice, paddy rice they have no water even dried and cracked. The cohesiveness of the community and improve the local wisdom in managing water resources greatly needed by the community, to improve the well-being so that development can proceed smoothly. All this is happening because they expect low levels of education, low income, lack of cultured environment, poor security environment and the low local wisdom.

Of the various problems above, I am interested in doing research on "The effect of educational level and local wisdom to the development of environment Nagari in Minangkabau".

## 2. Material and Methods

The research was conducted in Minangkabau. By taking a sample by purposive sampling area, the area chosen was Nagari Paringan, Nagari Sungai Antuan, Nagari Barung Barung Balantai Selatan, Nagari Sikucur and Nagari Paru. The population is a society that is in five Nagari above. While the sample is as many as 95 families.

The method used in this research is survey method with approach and multiple linear regression analysis. This study will examine or analyze the relationship between exogenous variables on endogenous variables, and measure its influence. Variables that researched there are five, namely: 1) Level of Education, 2) Local Wisdom and 3) Development Environment Nagari.

The population is all heads of families in NagariParingan, Nagari Sungai Antuan, NagariBarungBarungBalantai Selatan, Nagari Sikucurang Nagari Paru, while sample taken with simple random technique (Simple Random Sampling). The trials carried instruments to communities in NagariTabek as many as 30 heads of family, outside of the study sample.

This study uses the instrument as a tool, in the form of statement or statement is a translation of each of the existing indicators. Validity and reliability analysis is done in order to find a valid and reliable instrument in the required information.

The statistical analysis used in this study is the linear regression analysis and a double, to make it look the influence of exogenous variables on endogenous variable. The data processing used SPSS Version 22:00.

## 3. Result andDiscussion

### *3.1. Test Requirements Analysis*

*a. Normality test*

Test for normality using the Kolmogorov-Smirnov test technique (Test K-S) and as the basis for rejection or acceptance decision whether or not normal distribution of the data set at a significance level of alpha 0.05 (Table 1).

*b. Homogeneity test*

Homogeneity test is a test that is performed to see whether the data obtained from the sample homogeneous. Population variance homogeneity test carried out by Test homogeneity of variance. The results of calculations for test of homogeneity can be seen in Table 2.

This translates into significant value for each variable is greater than the significance level ($\alpha = 0.05$). Thus it can be interpreted that the distribution of data originated of a homogeneous sample.

### *3.2. Regression Analysis*

*a. Linear Regression of exogenous variables on endogenous variables*

F test is used to see the effect of exogenous variables on endogenous variables. With the help of SPSS version 22.00 in the following Table 3.

Table 1. Normality Test Results

| Variable | Significance | Alpha (α) | Description |
|---|---|---|---|
| Education level (X1) | 0,2 | 0.05 | Normal |
| Local Wisdom (X2) | 0,2 | 0,05 | Normal |
| Development Environment Nagari (Y) | 0,2 | 0,05 | Normal |

Table 2. Homogeneity Test Results

| Variable | Significance | Alpha (α) | Description |
|---|---|---|---|
| Education level (X1) | 0,162 | 0,05 | Homogen |
| Local Wisdom (X2) | 0,067 | 0,05 | Homogen |

Table 3. Effect of Environmental Education Development A gainstNagari (Table Model Summary[b])

| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate | Change Statistics | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | R Square Change | F Change | df1 | df2 | Sig. F Change |
| 1 | .327[a] | .107 | .097 | 13.993 | .107 | 11.153 | 1 | 93 | .001 |

Table 4. Effect of Local Wisdom Development Environment Nagari (Model Summary[b])

| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate | Change Statistics | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | R Square Change | F Change | df1 | df2 | Sig. F Change |
| 1 | .669[a] | .447 | .441 | 11.008 | .447 | 75.294 | 1 | 93 | .000 |

Table 5. Effect of Education and Local Wisdom A gainst Environmental Development Nagari (Model Summary[b])

| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate | Change Statistics | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | R Square Change | F Change | df1 | df2 | Sig. F Change |
| 1 | .692[a] | .478 | .467 | 10.754 | .478 | 42.159 | 2 | 92 | .000 |

The magnitude of the effect of educational level on the development of environment Nagari is 0.107 or 10.7% comes from the influence of education level. It is necessary to increase public education both formal, informal and non-formal to improve environmental development Nagari. Formal education can be derived by the public from the continuing schools to a higher level, non-formal education can people get from counseling, training, mass media at the hearing and which are on watch, while informal education can people get from counsel, pituah of NiniakMamak, see others practicing something. The level of education can increase, it is expected that environmental development Nagari will also increase. Public awareness is also in demand for it.

The magnitude of the effect of educational level on the development of environment Nagari is 0.447, meaning that 44.7% comes from the influence of local wisdom (Table 4). This can be increased again through increase public knowledge about the environment, public confidence in managing the environment, understanding/ knowledge society about the environment, customs/ ethics community in exploiting the environment.

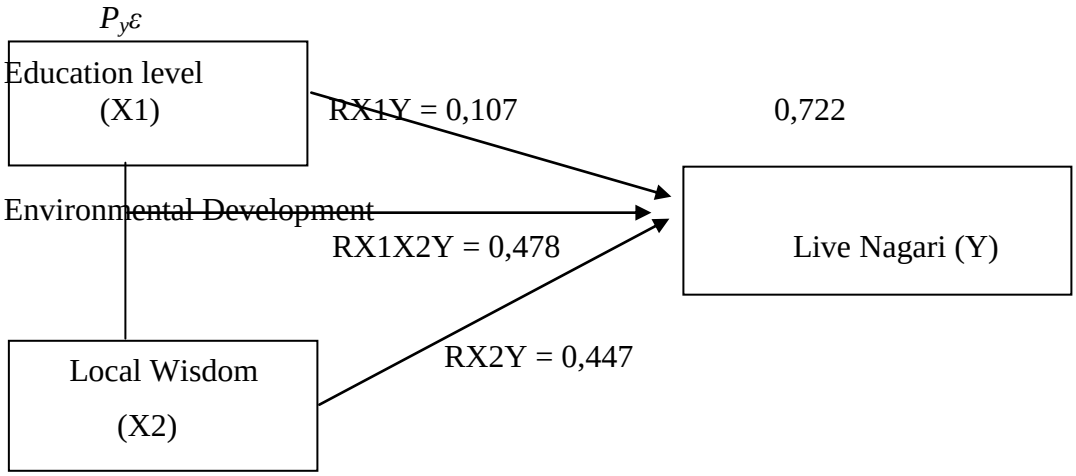


Fig. 1 The structure of the endogenous effects of exogenous variables.

*b. Multiple Regression of exogenous variables on endogenous variables*

The magnitude of the effect of educational level on the development of environment Nagari is 0.478, meaning that 47.8 % of the effect comes from the level of education and relation of 0.692 (Table 5).

Calculation of other variables not included in the model with the formula proposed by Sitepu (1994: 23) is as follows:

$$PYs = \sqrt{1 - R2YX1X2}$$
$$= \sqrt{1 - 0,478}$$
$$= \sqrt{0,522}$$
$$= 0.722$$

So the influence of other variables is equal to 0.478 (Table 5). So that the effect of variable levels of education, income level, cultured environment, environmental security, local knowledge on environmental development together amounted to 0.692, can be seen in the Fig. 1.

From the above results it turns out the effect of education on environmental development Nagari, compared with the influence of local knowledge on environmental development Nagari. It is caused by increased environmental development Nagari many influenced by local knowledge, because as we all know that local knowledge itua is a local policy that has been agreed the community to preserve the environment and leads people to behave towards the environment, so that the environment can be maintained sustainability. This is in accordance with the opinion of Keraf (2002) local wisdom is all forms of knowledge, belief, understanding or insight as well as custom or ethics that guide human behavior in life in ecological communities.

The level of education received by the public not only of formal education in schools, there will but education obtained from non-formal training, formal education acquired while in public from seeing, hearing or accept advice from NiniakMamak and AlimUlama in their Nagari.

Local knowledge Minangkabau society can be seen from NagariPariangan, Nagari Sungai Antuan, NagariSikucur, Nagari Barung Barung Balantai Selatan and Nagari Puru is the policy in each Nagari to preserve the environment, such as forest management, fish management ban and others. Local wisdom have already poured in Regulation Nagari but there are also local wisdom that has not entered into a code of Nagari. If already in a local wisdom NagariPernag means that already has the power to manage it.

Some documentation of indigenous communities in several Nagari as follows Fig. 2 to 6.

1. NagariPariangan

Fig. 2 Researchers at NagariPariangan Including Village rankings Most Beautiful In The World 2016

2. Nagari Sungai Antuan

Fig. 3 Researchers in Nagari Sungai Antuan, in JorongLubuakSimato manage springs from the hills into a clean water source community and surrounding

3. NagariSikucur

Fig. 4 Researchers at NagariSikucur manage IkanLarangan, 2016

4. NagariBarungBarungBalantai Selatan

Fig. 5 Documentation: Researchers at Nagaribarungbarung South Balantai, 2016

5. NagariParu

Fig. 6 Researchers at NagariParu manage HutanLarangan, 2016

## Conclusion

Based on the results of research and discussion, the conclusion are:

1. There is a degree of influence on the development of environmental education in MinagkabauNagari 10.7 %.
2. There is a degree of influence on the development of environmental local wisdom in MinagkabauNagari 44.7 %.
3. There is the influence of education level, local wisdom together towards environmental development in Minangkabau amounted to 47.8 %.

## References


[1] Badan Ketahanan Pangan. 2010. *Satu Dasawarsa Kelembagaan Ketahanan Pangan di Indonesia* (1999-2009). Jakarta: Kementerian Pertanian RI

[2] Badan Pusat Statistik. 2009. *Pengukuran Tingkat Pendapatan*. Jakarta: BPS

[3] David Krech, Richard S. Crutchfield, danEgerton L. Ballachey. 1996. SikapSosial (Judulasli: *Social Attitudes*). Jakarta: DepartemenPendidikandanKebudayaan Jakarta

[4] Geertz, Clifford. 1973. *The Interpretation of Cultures*. New York: Basic Books, Inc., Publishers

[5] Keraf AS. 2002. *Etika Lingkungan*. Jakarta : Penerbit Buku Kompas

[6] Koentjaraningrat(2000).*MasalahKesukubangsaan dan IntegrasiNasional*,Jakarta : UIP

[7] Pawarti, Amin, dkk, 2012. *Nilai Pelestarian Lingkungan dalam kearifan lokal Lubuk Larangan Ngalau Agung di Kampung Surau Kabupaten Dhamasraya Provinsi Sumatera Barat.* Prosiding. Semarang. UNDIP

[8] Sugiono (2012). *MetodePenelitianKombinasi (Mixed Methods).* Bandung: Alfabeta

[9] Sugiono (2014). *MetodePenelitianManajemen, PendekatanKuantitatif, KualitatifKombinasi (Mixed Methods)*,PenelitianTindakan (Action Research), PenelitianEvaluasi. Bandung: Alfabeta

# Local Food Development from Combination *Siarang* Variety of Black Rice (*Oryza Sativa L.Indica)* and Yellow Pumpkin *(Cucurbitamoschata)* to Prevent Anemia for Pregnant Women

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## Abstract

Incidence of anemia due to iron deficiency, especially in pregnant women is one of the biggest problem and difficult to overcome. Giving i*(Cucurbitamoschata)*ron supplement less favored by pregnant women because of the side effect of nausea. It required an innovative functional food that is rich of iron to prevent anemia in pregnant women. The purpose of this study was to develop a functional food that was made from a combination of *Siarang* black rice variety (*Oryza sativa* L. Indica) and Yellow Pumpkin *(Cucurbitamoschata)*, where these two materials are local food from Solok, West Sumatra, Indonesia. Food combination processed in the form of biscuits with 4 ratio of black rice and pumpkin respectively A (150:25), B (150:50), C (150:100) and D (150:150). Functional food biscuits were evaluated based on the content of nutrients and preference level of panelists on organoleptic performance of biscuits. The results showed that the most preferred type was biscuit D in terms of color, taste, aroma, and texture. Biscuit D also has contained highest iron level in the amount of 6.85 mg /100g and other nutrient compounds: fiber (6.12/100g) and vitamin C 8.25/100 g.

Keywords: anemia, black rice, pumpkin, functional food

## 1. Introduction

Anemia is one of malnutrition diseases that often foundand become the main nutritional problem in Indonesia [1]. Anemia can be defined as a condition where the hemoglobin (Hb) in the blood is less than normal, which is different for each age group and gender. Iron deficiency anemia is one form of malnutrition which is an important public health problem in worldwide, particularly in developing countries, including Indonesia. Consumption of iron from food is often lower than two-thirds of the adequacy of recommended iron intake, and the composition of the diet consumed belonging to the typeof food that are low absorption of iron [1].

Pregnant women are one of the most vulnerable groups to malnutrition because they have increased need to fulfill nutrional supply for mother and fetus. A non-observed diet in pregnant women will cause nutritional disorders such as anemia [2]. Anemia in pregnancy is one of the biggest nutritional problem and difficult to overcome throughout the world [1]. The prevalence of pregnant women are anemic in the world reach 41.8%, in developing countries reach 52%, in Southeast Asia the prevalence in preschool and pregnant women are estimated to be between 50% and 70% [3].

The Data of Indonesian Demographic and Health Survey (IDHS ) in 2010 stated that the maternal mortality rate (MMR) in

Indonesia was 220 per 100,000 live births. The numberwas still far from the target of the Medium-Term Development Draft in 2014 that was amounted to 2118 per 100,000 live births and the target of the Millennium Development Goals (MDG's) of 102 per 100,000 live births [3].

According to the WHO, a hemoglobin level less than 11.0 g / dl indicates the anemic condition in pregnant women [3]. The occurrence of anemia in majority pregnant women is caused by increased need during pregnancy for the nutritional compound in food and changes in the blood. The blood volume in pregnancy increases while the increased volume was not accompanied by the increase in the number of red blood cells. Iron deficiency in pregnancy is mainly caused by inadequate intake. To address the incidence of anemia among pregnant women, the government through the Department of Health implements a program providing iron tablets reaching 80% [3].

Based on the results of Basic Research (Riskesdas) in 2013, the prevalence of anemia in pregnant women in Indonesia amounted to 37.1%. Although the government hadthe anemia prevention program in pregnant women by giving 90 Fe tablets to expectant mothers during pregnancy in order to decrease anemia rate of pregnant women, butthe incidence of anemia wasstill high [3]

According to data from the monthly health report of Maternal and Child of Health Department ofWest Sumatra in 2011, the incidence of anemia in West Sumatra province amounted to 24.73%. Based on data from the monthly report of Padang Pariaman District Health Office had 13.3% of pregnant women are anemic. The incidence of anemia in Puskesmas Sicincin was high at 17.5% [3].

Giving iron supplement and nutritional improvement is an important effort in the anemia prevention. Iron supplementation for pregnant women has been started since 1974, but the results werenot satisfactory. Weakness in consuming Fe tablet to overcome anemia in pregnant was the side effects that such as nausea and vomiting that make pregnant women are lazy to consume the tablet, so that there is a necessary to preventanemia by alternative way such as the use of foodstuffs [3].

One of foodstuffs that can prevent anemia in pregnant women is*Siarang* black rice variety *(Oryzasativa*L.Indica) that has a low protein and highiron content 15.52 mg / 100 g in the form of biscuits.Siarang black rice is a local variety fromGumanti valley districts, Solok regency. In the last few years popularity of black rice increased and consumed as a functional food or only used for traditional food area without knowing the nutrient content in the black rice (Siarang) which have benefits for health, especially prevention of anemia in pregnant women due to the high content of iron [4 ].

Black rice is cultivated organically by PPO Santiago in Sariak Alahan Tigo Nagari District of Hiliran Gumanti and black rice production is sold in the Talang Babungo market, Hiliran Gumanti District, and Alahan Panjang market, District of Lembah Gumanti. The potential nutrients (rich of Iron and Vitamin C) and the availability of pumpkins in Indonesia are plentiful, then the effort to diversify the pumpkin to be a functional food needs to be done for example by adding pumpkin into black rice biscuit. Biscuit is one of the processed pastry products which are favored snacks because of delicious, sweet and crunchy and has a long shelf life [5].

The selection of yellow pumpkin as an additional compound in Siarang black rice biscuit because of the content of iron and vitamin C in pumpkins higher than other foodstuffs such as red sweet potato, brown rice, and squash. The addition of pumpkin on preparation of a black rice biscuit was expected to be another alternative in acquiring the intake of iron (Fe) and will increase public acceptance of the black rice biscuits.

Table 1. Comparison of pumpkin with black rice

| Samples | Comparison (g) | |
|---|---|---|
| | **Black rice** | **Pumpkin** |
| A | 150 | 25 |
| B | 150 | 50 |
| C | 150 | 100 |
| D | 150 | 150 |

This research is early stage of the use of Siarangblack rice (*Oryza sativa* L. Indica) and pumpkin *(Cucurbita moschata)* as a functional food for anemic pregnant women.

## 2. Material and Methods

### *2.1. Materials*

Materials and tools used for biscuit preparation are Black rice fluor, Yellow pumpkin fluor, eggs,Margarine ,salt, baking powder, oven,plates,Blender, container, mixer, Spatula scales, and linen cloth.

Materials and Tools used for quality evaluation of biscuits areHCl, $H_2SO_4$, bromophenol blue, sodium acetate, aquadest, Selenium, $KIO_3$, drop pipettes, standard chemical glasses and Kjedahl flask.

### *2.2. Research methods*

This study was experimental, using a completely randomized design (CRD) with 3 treatments with and repetitions. The treatment with the addition of pumpkin on black rice in the manufacture of biscuits. Comparison can be seen in Table 1.

### *2.3 Making Black Rice Flour and Yellow Pumpkin Flour*

Black rice sieved to remove debris such as gravel, chaff and grain. Black rice thoroughly washed first, then soaked in water for 6 hours. After that black rice was drained and dried so that resulting moist rice. Furthermore, moist rice was finely ground using a hammer mill grinder with 80 meshsize. Moist rice was more easily smoothed so that the milling process faster and energy efficient. Once milled rice flour need to be dried or dried to a moisture content below 14%.

### *2.4 The process of making yellow pumpkin flour*

Pumpkin was cut longitudinally prior to pieces. Then, washed with water to drain and remove dirt attached to the skin of the fruit, washing was done until the pumpkin skin really clean. Peeling pumpkin washed subsequently removed the seeds and fibers, and peeled it clean. Pumpkins were peeled cut into thin slices and small with the aim to accelerate the drying process. Drying could be done by using solar power (exposing) for 4- 6 days (depending on weather). Drying yellow pumpkins were immediately milled or destroyed using flour grinding tools, grinding was done until the pumpkin dry crumbled into powder (flour) and then sieved to 60 mesh sieve holes. If the sieved pumpkin couldn't pass the sieve it could be milled again until it can passed the sieve. Utilization after completion of sieved flour ready to be processed.

### *2.5. Making Biscuits*

Biscuit-making procedure is as follows: The materials prepared according to a predetermined formula. eggs, powdered sugar, margarine, whipped with a mixer until fluffy for 15 minutes, then mixing flour (flour Black rice flour and pumpkin), vanilla, *baking powder* incorporated in the dough after it was printed and baked in the oven 155$^0$C for 15 minutes later into biscuits.

## 3. Result and Discussion

Based on the results of organoleptic tests showed that the best samplewas code D (150 g black rice: pumpkin 150 g) with good criteria. There are four indicators were assessed at the organoleptic test which included color, texture, aroma and taste.

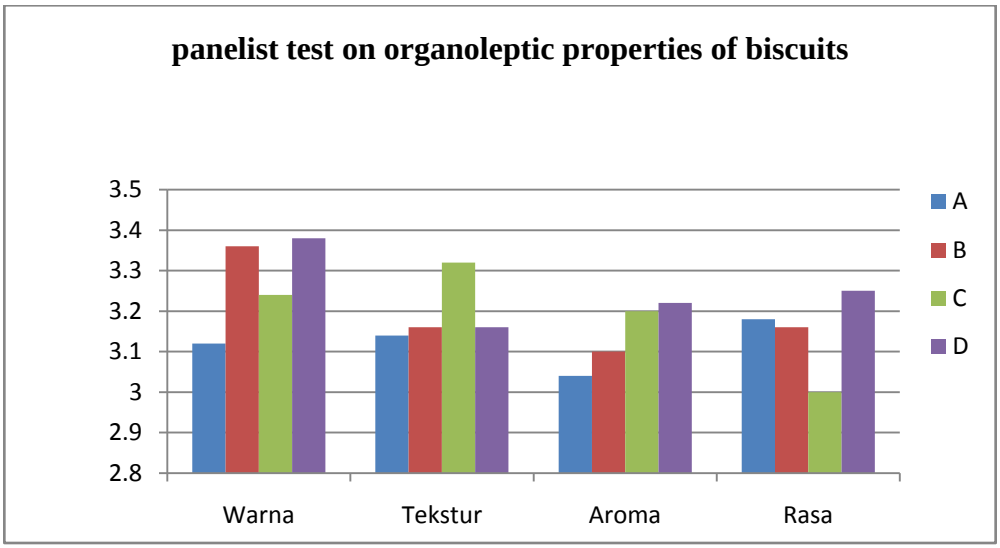


Fig. 1 Graph of favorite test of panelists on organoleptic properties of biscuits

Table 2.Nutritional Content of Sampel Biscuits

| Samples | Iron Levels mg/100 g | Fiber levels (mg/100 g) | Vitamin C levels (%) |
|---|---|---|---|
| A (150 : 25) | 2,98 | 2, 25 | 6,24 |
| B (150 : 50) | 3,72 | 3, 67 | 7,11 |
| C (150 : 100) | 5,22 | 3, 85 | 7,98 |
| D (150 : 150) | 6,85 | 6, 12 | 8,25 |

The best color combination of black rice biscuits and pumpkin wereyellowish brown color of sample D. Yellowish brown color was also seen in the biscuitsamples B and C. Yellowish brown color was obtained from a combination of black rice and pumpkin. Lowest average value was found on sample A that had small pumpkin addition in combination with black rice and lesser panelists who like the sample.

Yellowish colour of biscuits was yield from pumpkin colour. The pumpkins contain high carotene, lutein, zeaxanthin giving yellow colour of pumpkins that help to protect the body by neutralizing oxygen molecules called free radicals [6].

The results showed that the sample D (150 g black rice: pumpkin 150 g) was the preferred sample by panelists. Samples with a balance combination of black rice biscuits and pumpkin were produced savory flavors.That was influenced by the addition of pumpkin affecting flavor biscuits produced. The texture and consistency of the material would affect the flavor posed by the material [6] The treatment gave the impression of a specific taste for all type of biscuits that had a different flavor. The higher use of pumpkin gave the impression of a distinctive sweet taste of pumpkin.

Based on the results of nutritional compound evaluation of biscuit samples found that sample D (150 :150) was the best formula biscuit. Sample contain higher iron (Fe), fiber and vitamin C higher than other type of biscuits. Nutritional compounds increased and appropriated to amount of pumpkin addition. The higher pumpkin addition, the higher iron levels in biscuits. Black rice contains fiber food in its aleuron [7]. Fiber or so-called dietary fiber is very good for health, high fiber content in the

steamed cakes of black rice flour is very beneficial for the body, especially the digestive health and the health of other organs [8]. Vitamin C is good for improving the health of pregnant woman's body.

## Conclusion

Biscuits made from local foodstuff had a potency as an alternative diet for pregnant women to overcome anemia. Pumpkin addition to Siarang black rice biscuits in combination 150:150 (sample D) had a good organoleptic performance, more panelists like and contained highest Fe, fiber and vitamin C level compared to other samples.

## References

[1] Manuaba. 2010. *Ilmu Kebidanan Penyakit Kandungan dan KB.* Jakarta : EGC

[2] Proverawati, Atikah. 2011 .*Anemia dan Anemia Kehamilan.* Yogyakarta : NuhaMedika

[3] Kemenkes RI., 2013. *Profil Kesehatan Indonesia Tahun* 2013. Jakarta. Http://www.depkes.go.id/download/PROFIL_KESEHATAN_INDONESIA*2013.pdf :* di akses 15 Oktober 2015

[4] Suardi, D. and I. Ridwan. 2009. *Beras hitam, pangan berkhasiat yang belum populer. Warta Penelitian dan Pengembangan Pertanian* 31(2): 9-10.

[5] Koswara, Sutrisno. 2006. *Krekerss dan Cookies*. Available at hhtt://www.ebookpangan.com/ARTIKEL/Krekers dan Cookies. Pdf. (13 Agustus 2009).

[6] Winarno, F. G. and A. Rahman, 2008. *Pengantar teknologi Pangan*. Gramedia. Jakarta.

[7] Monika, Pricilia. 2013. *Aktivitas Antioksidan Beras Organik Varietas Lokal (Putih Varietas Cianjur, Merah Varietas Saodah, Hitam Varietas Jawa).* Seminar Nasional: Menggagas Kebangkitan Komositas Unggulan Lokal Pertanian dan Kelauatan Falkultas Pertanian Trunojoyo Madura.

[8] Nainggolan and Cornelis Adimunca. 2005. *Diet Sehat Dengan Serat.*Cermin Dunia Kedokteran No. 147, 2005 Depertemen Kesehatan RI, Jakarta.

[9] Dalimartha, Setiawandan Adrian, Felix. 2010. Khasiat Buah dan Sayur. Jakarta : Penebar Swadaya.

# Development of Antimicrobial Analysis of Lactic Acid Bacteria Isolated from VCO (Virgin Coconut Oil) Fermentation Process Against Bacteria in The Secretion of CSOM (Chronic Suppurative Otitis Media) Patient

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## Abstract

The aim of this study is to develop the antimicrobial and antifungal analysis of Lactic Acid Bacteria (LAB) in VCO (Virgin Coconut Oil) process, they are: *Lactobacillus plantarum, Lactobacillus thermobacterium, Corineaebacterium bovis, Corineaebacterium xerosis* and *Micrococcus luteus.* Antimicrobial and antifungal analysis was conducted using pathogenic bacteria (*Eschericia coli, Staphilococcus aeureus, Bacillus subtilis, Listeria monocytogenes,* and *Salmonella thypi) and pathogenic fungi* (*Candida sp* and *Aspergillus sp).* LAB can inhibit the growth of those pathogenic bacteria and fungi. Because LAB in VCO can inhibit the growth of pathogenic bacteria, we assume that LAB also can inhibit the growth of pathogenic bacteria in CSOM (Chronic Suppurative Otitis Media) patient that can cause brain abscess, meningitis, and even death. Research methods used in this research are (1) Isolate the pathogenic bacteria and fungi in the secretion of CSOM patient using Blood agar and dilution method; (2) Identify the isolates of pathogenic bacteria and fungi morphologically, using gram-test and other biochemical tests. From 60 secretions of CSOM patients, there were 87 isolates obtained. 82 (94,3%) isolates consist of *Staphylococcus aureus, Psedomonas aureginosa, Klebsiella sp* and *Proteus mirabilis* and 5 (5,7%) isolate from fungi *Candida sp.*

Keywords: Pathogenic bacteria, lactic acid bacteria, virgin coconut oil

## 1.Introduction

Lactic Acid Bacteria (LAB) are bacteria exist in fermentation process that contain high carbohydrates, they also exist in coconut milk which if it is fermented, it will produce Virgin Coconut Oil (VCO) [1], [2], [3]. Beside LAB have successfully fermented coconut milk to VCO, LAB in VCO fermentation process were successfully isolated and obtained 187 isolates that were identified as; *Lactobacillus plantarum, Lactobacillus thermobacterium, Corineaebacterium bovis, Corineaebacterium xerosis* and *Micrococcus luteus.* In LAB, there are bacteriocins that are nucleotide to inhibit the growth of pathogenic bacteria, but they are not dangerous for non-pathogenic bacteria [4]. One of bacteriocins in LAB of VCO fermentation process that was successfully isolated and analyzed was *Lactobacillus plantarum* NM 178-5. The antibacterial and antifungal analysis of this bacterocins were successfully conducted [5]. It turns out the LAB can inhibit the growth of 5 samples of pathogenic bacteria which are; *Eschericia coli, Staphilococcus aeureus, Bacillus subtilis, Listeria monocytogenes, Salmonella thypi,* and also 2 samples of pathogenic fungi which are; *Candida sp* and *Aspergillus sp.*

Pathogenic bacteria are very dangerous for our health like the pathogenic bacteria in the secretion of CSOM patient. In more

serious level, the disease can cause brain abscess, meningitis, deafness, even death [6]. Pathogenic bacteria in the secretion of CSOM patient are *Psedomonas aureginosa 40%, Staphilococcus, E.coli 12%, Klebsiella 5%*, and *Proteus 5 %s* [7]. Those pathogenic bacteria are usually sensitive toward antibiotic. Prakash (2013) found 41,25 % *Staphilococcus aureus*, 5% *E.coli*, 7,5 % *Klebsiella* , 5% *Proteus sp* and 37,5% *Pseudomonas sp.* as the pathogenic bacteria in the 80 secretions of CSOM patients.

Based on the similarity between pathogenic bacteria in the secretion of CSOM patient and pathogenic bacteria that are used as the sample on antibacterial and antifungal analysis of LAB in the oil layer of VCO, we hope LAB can inhibit all pathogenic bacteria in CSOM patient. This is supported by the report of Prayaga (2013) that said there are pathogenic bacteria that resistant toward few antibiotics such as ciprofloxacin. Bakhshaee and Rajati (2010) also found that few pathogenic bacteria that infected CSOM patient are resistant as well [8]. Therefore, LAB from VCO can be used as natural antibiotic. The aim of this research in long term is to produce VCO that can be natural antibiotic which can be dripped into the patient's ear. Meanwhile, the aims of this research in short time are; to isolate the pathogenic bacteria in the secretion of CSOM patients as the samples to ensure what kinds of pathogenic bacteria exist in the secretion of the patients; to identify the bacteria morphologically, gram-test, biochemical tests, or physiological test; and to do molecular identification to ascertain the type of pathogenic bacteria.

## 2. Material and Method

This research was conducted from April until October 2016. The secretions were taken from 103 CSOM patients in various hospitals in West Sumatra. Data analysis was conducted in Central Laboratory of X Hospital, Basic Laboratory of Kopertis X, Microbiology Laboratory of Muhammadiyah University of West Sumatra, and Microbiology Laboratory of LIPI Cibinong.

### *2.1.Material*

Material in this research was ear liquid of 103 CSOM patients in X Hospital. The media to grow the bacteria during conventional isolation and identification processes were Blood agar and McConkey agar. MRS (15g peptone, 5g yeast extract, 10g dextrose, 5g tomato juice, 2g monopotassium phosphate, and 1g polysorbate 80), Luria-Bertani medium (10g tryptone, 5g yeast extract, and 10g NaCl), sodium acetate, liquid nitrogen, methylene blue, sterile aquadest, sodium azide, HCl 6 N, ampicilin, ammonium sulfate, Tris-HCl 50 mM pH 7.4, NaCl 1 M, Tris-HCl 100nM pH 8.5, glycerol, isopropanol, 70% ethanol, ammonium molybdate, trisodium citrate, aquabidest, methanol, pure Agar, 70% alcohol, 96% ammonium sulfate (NH4)2SO4, Aquadest, buffer solution pH 7, technical hydrogen peroxide (H2O2), potassium ydroxide (KOH), phenolphthalein (PP) analysis, technical starch, lactose broth, and thioglycolic acid.

### *2.2.Method*

There were 3 stages in this research; (1) isolation of pathogenic bacteria in the secretion of 126 CSOM patients; (2) Identification of pathogenic bacteria using gram-negative and positive test, bacterial staining test, morphology test, and biochemical test such as catalase test and other carbohydrate tests; (3) Molecular identification by analyzing RNA 16S rDNA using PCR.

#### *2.2.1. The Isolation of Pathogenic Bacteria*

We did isolation stage before doing the identification of pathogenic bacteria in the secretion of 60 CSOM patients. Pathogenic bacteria from 60 CSOM patients were isolated using dilution method until $10^{-7}$ dilution level and the media used to isolate the pathogenic

bacteria were blood agar and Mac Conkey agar. Streak the bacteria for a single colony and it will be the isolate of the pathogenic bacteria. Along with the secretion in blood agar, the secretion was also enriched in thioglycolate. If there was no bacterium grew in the media, we can take the sample that has been already enriched and growth in blood agar. Usually, CSOM patient produce one isolate, but there are few patients that can produce more than one different isolate.

*2.2.2. The Identification of Isolate of Pathogenic Bacteria*

Isolates that have been collected were identified morphologically by seeing the colony pattern, and the color of the colony. Positive and negative-gram test, biochemical test such as catalase test, starch test, and novobiocin test were also conducted.

## 3. Result and Discussion

In this research, the distribution of the CSOM patients that used as samples can be seen in Table 1 below:

From Table 1, we can see that 68.3% of CSOM patients in this research are children, and 31.6% are adults. This finding is supported by Mansoor T. et al (2009) in their previous research that found CSOM can attack children and also adult. Sample distribution in their research were 28% aged 11-20, 17% aged 21-30, 5% aged 31-40, 3% aged 41-50, 4% above 51, and there were few patients than cannot be predicted the age [9]. In this research, we can see most of the patients are children, but the age categorization in previous research was more complicated. Shresta (2011) reported that most of CSOM patients were children. 34.8% of them under 10, 30.4% aged 11-20, 6.5% above 41 [7]. In other research, Shresta (2012) found that 146 of CSOM patients in his research were children under 20 years old, 19% aged 21-40, and 8% above 40 [10].

Table 1. Sample Distribution

| Patient | Number of samples | % |
|---|---|---|
| Children (under 13 years old) | 41 | 68,3 |
| Adult (above 20 years old) | 19 | 31,6 |
| Male | 47 | 78,3 |
| Female | 13 | 21,6 |

Children are easy to be attacked by this disease because of the poor hygiene practice and swimming. Most of children are afraid to tell their parents or family about it.

From Table 1 we also can see that most of the CSOM patients are 78.3% male, and 21.6% female. This is supported by Shyamala (2012) that found 57% of the CSOM patients in his research were male, and 43% of the were female [10]. Yaor and Jafari (2006) also found that 41 CSOM patients were male, and 32 CSOM patients were female [11]. Meanwhile, Shresta (2011) found that male patients (44.8%) were less than female patients (55.2%) [7]. Prakash also has reported before that in Nepal, most of the CSOM patients were male. 54% of the patients were male and 46% were female. It is caused by hygiene factor. As we know that men pay less attention to cleanliness than women [12].

*3.1. The Isolation of Pathogenic Bacteria*

From pathogenic bacteria isolation in this research, we obtained 87 isolates from 60 secretions of CSOM patients. Although we usually find 1 isolate in every secretion, but there is secretion of CSOM patient that contains more than 1 isolate of pathogenic bacteria. Suryani (2016) found 42 isolates of pathogenic bacteria in 42 secretions of CSOM patients [13]. But, Shresta (2011) found 232 isolates of pathogenic bacteria in 230 secretions of CSOM patients. It means that there are 2 patients that contain 2 isolates in their secretions [7].

Table 2. Morphological Analysis of the Isolates

| Macroscopic Characteristics of Isolate | Isolate | Number of Isolates |
|---|---|---|
| - The color is grayish white<br>- The shape like a fragment<br>- The size is 6-15 mm<br>- The texture is rough<br>- Greenish pigment<br>- Smelly<br>- Gram-negative (bacilli) | *Pseudomonas aureginosa* | 74 (58,7%) |
| - Circular shape<br>- The size is medium<br>- Convex<br>- Possessing flagella<br>- Spread<br>- Smell salty<br>- Gram-negative (bacilli) | *Proteus mirabilis* | 21 (16,6%) |
| - Circular shape<br>- The size is big<br>- Convex<br>- Mucoid<br>- Shiny<br>- The edge is smooth<br>- Gram-negative bacilli | *Klebsiella* | 7 ( 5% ) |
| - Circular shape<br>- Slightly Convex<br>- The edge is smooth<br>- The color is yellowish white<br>- The size is 2-5 mm<br>- β hemolytic<br>- Positive-gram (cocci)<br>- Aciniform (Grouped like grapes) | *Staphylococcs aureus* | 14 (11 % ) |
| - Circular shape<br>- Slightly Convex<br>- The edge is smooth<br>- The color is white<br>- The size is small<br>- Cocci<br>- Positive-gram<br>- Aciniform (Grouped like grapes) | *Staphylococcus epidermidis* | 4 (3 %) |

Table 3. Morphologic Analysis of Fungi from the isolates of Pathogenic Bacteria

| Characteristics | Isolate | Number of Isolate |
|---|---|---|
| - Positive-gram<br>- Pseudohypha +<br>- Didn't grow in blood agar<br>- Grow in saboraud<br>- Circular shape, white, and slightly mucoid | *Candida sp* | 6 (4,7%) |

Table 4. Result of Biochemical Test of the Isolate

| Test | Result | Number of Isolate | Isolate |
|---|---|---|---|
| TSIA | K/K | | |
| Gas | + | | |
| H2S | _ | 52(58,7%) | *Pseudomonas aureginosa* |
| SC | + | | |
| Sulfur | + | | |
| Indole | - | | |
| Motile | + | | |
| TSIA | K/A | | |
| Gas | + | | |
| H2S | + | 15(17,24%) | *Proteus mirabilis* |
| SC | + | | |
| Sulfur | + | | |
| Indole | - | | |
| Motile | + | | |
| TSIA | A/A | | |
| Gas | + | | |
| H2S | - | 5(5,7%) | *Klebsiella* |
| SC | + | | |
| Sulfur | - | | |
| Indole | - | | |
| Motile | - | | |
| Catalase | + | | |
| Gas | + | | |
| Coagulase | + | 10(11,49%) | *Staphylococcus aureus* |
| Novobiocin | Sensitive | | |
| Catalase | + | | |
| Gas | + | | |
| Coagulase | - | 2(3%) | *Staphylococcus epidermidis* |
| Novobiocin | Sensitive | | |

Mansoor *et al* (2009) also found that usually there is one isolate of pathogenic bacteria in every secretion [9]. But in this research, we found 275 isolates of pathogenic bacteria in 263 secretions of CSOM patients. It means that there are patients that have more than one isolates.

### *3.2. Morphological Identification of Pathogenic Bacteria*

Morphological identification result of pathogenic bacteria in 60 secretions of CSOM patients is described in Table 2. Meanwhile in fungi identification, when we were doing observation of isolation process, we found a

colony that had hypha in it. We did gram staining test and the result was positive pseudohyphae. Then, the samples were growth in blood agar and saboraud agar. The colony did not grow in blood agar but grew in saboraud agar in circular shape, white, and slightly mucoid. The result can be seen in Table 3.

*3.4. Biochemical Test*

The result of biochemical test of the isolates can be seen in Table 4. From the result of morphology identification in Table 2 and 3, we can see the shape, color, size of colony from each isolate and also the gram test result. From all result above, the pathogenic bacteria in the secretion of CSOM patients in X Hospital are *Pseudomonas aureginosa* (58,7%), *Staphilococus aureus* (11,49 %), *Staphilococus epidermidis* (3%), *Proteus mirabilis* (17.24%), *Klebsiela sp* (5,7%) and 1 fungus *Candida sp* (1,1%).

This result is supported by the result of biochemical test in table 4. This matter also has been reported by other scientists but there were a few differences about the pathogenic bacteria and fungi found in the secretion of CSOM patients.

Mansoor et al (2009) said that they only found pathogenic bacteria and didn't find fungi in the secretion of CSOM patient. He found bacteria that also were found by other researchers. They are *Pseudomonas aureginosa, Staphilococus aureus, Staphilococus epidermidis, Proteus mirabilis, and Klebsiela sp* and he found *Streptococcus* and *E.coli* that were not found by other researchers [9]. Meanwhile we found fungi *Candida sp* in this research. Prakash et al (2013) also found pathogenic bacteria only and didn't find fungi. The bacteria were *Staphylococcus aureus* , *Pseudomonas sp, E.coli, Klebsiella* and *Proteus sp* [15]. Moorthy et al (2013) only found 2 pathogenic bacteria, they are *Pseudomonas aureginosa* and *Staphylococcus aureus* [14]. *Pseudomonas aureginosa and Staphilococus aureus* are the common bacteria found by the researchers in the secretion of CSOM patients.

## Conclusion

From all result above, we can conclude that the number of isolates of pathogenic bacteria obtained in the secretions 60 CSOM patients were 87 isolates. And there were 5 kinds of pathogenic bacteria; *Pseudomonas aeruginosa, Klebsiella, Proteus, Staphylococcus aureus* and also 1 kind of fungi called *Candida spp* that was identified morphologically, gram test and also other biochemical tests. To perfect the future research related to this topic, we recommend other researchers to ensure the kind of bacteria and fungi obtained by identifying them molecularly using 16S rRNA analysis and do the sequent of the DNA and compare them with the data in DDBJ.

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## Reference

[1] S. Djajasoepena, O. Suprijana, and M. Resmelia, "Virgin coconut oil production by fermentation using Saccharomyces cerevisiae," vol. 2011, no. November, p. 19413, 2011.

[2] S. Redjeki and E. Kurniati, "The Kinetic Reacion of Virgin Coconut Oil (VCO) Fermentation in an Ideal

Bioreactor Tank in a Batch Process," vol. 7, pp. 159–163, 2013.

[3] A. Suryani, Dharma, Y. Manjang, S. Arief, E. Munaf, and N. Nasir, "Research Journal of Pharmaceutical , Biological and Chemical Sciences Antimicrobial and Antifungal Activity of Lactic Acid Bacteria Isolated from Coconut Milk Fermentation .," vol. 5, no. 1587, pp. 1587–1595, 2014.

[4] B. S. Nurul-iman, Y. Kamisah, K. Jaarin, H. Mohd, and S. Qodriyah, "Virgin Coconut Oil Prevents Blood Pressure Elevation and Improves Endothelial Functions in Rats Fed with Repeatedly Heated Palm Oil," vol. 2013, 2013.

[5] D. A. Suryani, "Isolation and Characterization of Bacteriocins Bacteria Lactobacillus Plantarum Strain NM178-5 from Fermentation Process with Contained on Coconut Milk," *Transylvanian Reviwer*, vol. XXIV, no. 6, pp. 614–628, 2016.

[6] H. M. Massa, A. W. Cripps, D. Lehmann, and M. Journal, "Otitis media: viruses, bacteria, biofilms and vaccines," vol. 191, no. 9, pp. 4–9, 2009.

[7] B. L. Shrestha, "microbiological profile of chronic suppurative otitis media" vol. 2, no. 2, pp. 6–7, 2011.

[8] M. Bakhshaee and M. Rajati, "Allergic rhinitis and chronic suppurative otitis media," 2010.

[9] T. Mansoor, M. A. Musani, G. Khalid, and M. Kamal, "Pseudomonas aeruginosa in chronic suppurative otitis media : sensitivity spectrum against various antibiotiCS," vol. 21, no. 2, pp. 120–123, 2009.

[10] Ps. R Shyamala, "The study of bacteriological agents of chronic suppurative otitis media - Aerobic culture and evaluation," *J. Microbiol. Biotechnol. Res.*, vol. 2, no. 1, pp. 152–162, 2012.

[11] M. A. Yaor and B. Jafari, "Surgical Management of Chronic Suppurative Otitis Media : A 3-year Experience," *Annnals African Med.*, vol. 5, no. 1, pp. 24–27, 2006.

[12] S. Prakash Adikari, "Chronic Suppurative Otitis Media in urban private school children of Nepal," *Braz. J. Otorhinolaryngol*, vol. 75, no. 5, pp. 2007–2010, 2009.

[13] A. Suryani, Dharma, Y. Manjang, U. Muhammadiyah, S. Barat, and U. Andalas, "Isolasi bakteri patogen pada pasien penderita infeksi telinga Chronic supparative otitis media ( OMSK )," *J. Katalisator*, vol. 1, no. 2, 2016.

[14] P. N. S. Moorthy, J. Lingaiah, S. Katari, and A. Nakirakanti, "Clinical Application of a Microbiological Study on Chronic Suppurative Otitis Media," vol. 2013, no. November, pp. 290–294, 2013.

[15] M. Prakash, K. Lakshmi, S. Anuradha, and S. Gn, "Bacteriological profile and their antibiotic susceptibility pattern of cases of chronic suppurative otitis media," vol. 6, pp. 5–7, 2013.

# The Influence of Dicamba in Combination with BAP on Callus Induction and Proliferation of Centella (*Centella asiatica* L.)

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## Abstract

*In vitro* culture is an alternative method for large-scale propagation of Centela (*Centella asiatica* L.) due to the increasing rarity of this plant. Previous study had proved that 4 mg/L Dicamba could induce and proliferate Centella calli, however, the result was still not optimum. Therefore, the addition of BAP into medium containing Dicamba in this current research was aimed to maximize the result of calli. Explants used in this research were leaves and petioles derived from sterile culture of centella var. Castina 3. The treatments consisted of Murashige and Skoog (MS) medium that was used as basic medium enriched with one concentration level of auxin (4 mg/L Dicamba) combined with various concentration levels of cytokinin (0, 0.1, 0.3, 0.5, 0.7, 0.9 and 1.1 mg/L BAP). Callus induction was observed at the fourth week of culture, after that the calli were subcultured on the same media formulation for proliferation that was observed at the eighth week of culture. The result revealed that the best media formulation for callus induction was 4 mg/L Dicamba combined with 1.1 mg/L BAP, which was indicated by the highest percentage of callus formation (100%) and fresh weight of calli i.e. 0.54 g and 0.48 g for leaf and petiole explants, respectively at the induction stage; whislt the fresh weight of calli at the proliferation stage was 1.21 g and 1.59 g for leaf and petiole explants, respectively. In addition the texture of callus produced from this medium was friable at the induction stage and friable compact at the proliferation stage.

Keywords: Dicamba, BAP, callus induction, proliferation, *Centella asiatica* L.

## 1. Introduction

Centella (*Centella asiatica* L.) is an important medicinal plant that has bioactive content in various medicinaly utilities. The compounds responsible for biological activity of centela are triterpenoid such as asiacotide, madecassoside, asiatic acid, and madecassic acid [1]. Concerning its biological activities make Centella as one of important raw materials in jamoe industry [2], [3], herbal medicine [4], [5], and cosmetic [6], [7], [8]. As a result the necessity and demand for centella continuously increase from time to time. Unfortunately, the effort to propagate of centella is still rarely and to fulfill the demand of raw materials for industry it is often harvested directly from nature. Those acts make the availability of the plant in nature is decreasing because of over exploitation without replanting [9], [10].

*In vitro* propagation of centella could be done by indirect regeneration technique through callus phase [11],[12]. Callus is widely used either for basic research or for the application in world of industry [13]. The advantages from this technique besides can be obtained seedlings in a large numbers with only using a small part of mother plant [11],

[14], it can also be used in callus culture and cell suspension culture to produce a secondary metabolite especially asiacotide that has important pharmacological activities in centella [15] [16].

In previous study, it was discovered that 4 $mgL^{-1}$ Dicamba was the best media formulation for callus induction of centella [17], although the quantity and quality of calli produced were still low. Therefore, in this research 4 $mgL^{-1}$ Dicamba was enriched with cytokinin such as BAP in various concentrations in order to generate more calli. Cytokinin activity is a key element in establishing the organization and regulating cell division with genetic studies indicating that cytokinin is a positive regulator of cell proliferation [18]. It is expected by the addition of BAP can increase the quantity and quality of callus resulted. In this research was also conducted subculture phase since sub culturing plays a vital role in accumulation of secondary metabolites [15]. The objective of this current research was to maximize the production of *centella calli*.

## 2. Material and Methods

The research was conducted at the Indonesian Center for Agricultural Biotechnology and Genetic Resource Research and Development (ICABIOGRAD), Indonesian Agency for Agricultural Research and Development (IAARD), from August to October 2016.

### *2.1. Plant Material*

Materials used for the culture were stolon of Centella variety Castina 3 which was planted in green house. Stolons were disinfected by 0.5 $gL^{-1}$ benomyl and 20 $gL^{-1}$ streptomycin sulphate for 1 hour, respectively. Subsequently, they were sterilized with 70% alcohol for 5 min, 15.75% sodium hypochlorite for 7 min and 10.5 % sodium hypochlorite for 9 min. They were rinsed with sterile aquadest three times. The apical shoots of the stolon were isolated. They were planted on MS (Murashige dan Skoog, 1962) medium without plant growth regulator and then incubated for 5 weeks.

### *2.2. Callus Induction and Proliferation*

For callus induction, leaves (± 0.5 x 0.5 cm) with the ventral side of the leaf explants facing the media and petioles (± 1 x 0.15 cm) were isolated from sterile cultures and planted on MS medium enriched with one types of auxins, namely Dicamba (3,6-dichloro-2-methoxybenzoic acid) at the concentration of 4 $mgL^{-1}$ combined with BAP at the concentration of 0, 0.1, 0.3, 0.5, 0.7, 0.9 and 1.1 $mgL^{-1}$. The same media formulation used for proliferation. The pH media was adjusted to 5.8. All media were added with 30 $gL^{-1}$ sugar and solidified with 2.5 $gL^{-1}$ phytagel. The cultures were incubated in the culture room with photoperiodicity for 16 h, temperature at 22$^{o}$C and light intensity of 300 lux. Every treatment consisted of 10 replications (bottle). Each bottle contained 1 explant.

Parameters observed were percentage of callus induction, fresh weight of callus, growth rate of callus, color and texture of callus. Score of callus growth rate were as follows:

0 = no callus growth
1 = callus growth on 1 – 25% part of explant
2 = callus growth on 26 – 50% part of explant
3 = callus growth on 51 – 75% part of explant
4 = callus growth on 76 – 100% part of explant

The observations for callus induction and proliferation were conducted at the fourth and eighth week, respectively. Data collected was analyzed with Analysis of variance (Anova) based on Fully Randomized Design. Least Significant Difference at 5% level was used to distinguish among treatments.

Table 1. Mean of fresh weight of callus on callus induction and proliferation

| Treatment | Induction | | Proliferation | |
|---|---|---|---|---|
| | Leaf Exp. | Petiole Exp. | Leaf Exp. | Petiole Exp. |
| Dic | 0.2519 a | 0.1300 a | 0.5781 a | 0.3804 a |
| Dic + 0.1 $mgL^{-1}$ BAP | 0.3578 abc | 0.2084 a | 0.7908 ab | 0.6203 a |
| Dic + 0.3 $mgL^{-1}$ BAP | 0.2942 ab | 0.3013 abc | 0.6086 a | 0.9458 b |
| Dic + 0.5 $mgL^{-1}$ BAP | 0.5205 cd | 0.2911 ab | 0.9949 b | 0.9739 b |
| Dic + 0.7 $mgL^{-1}$ BAP | 0.4647 bc | 0.3861 bc | 0.9632 b | 1.0453 b |
| Dic + 0.9 $mgL^{-1}$ BAP | 0.5449 d | 0.4826 c | 1.2147 c | 1.5854 c |
| Dic + 1.1 $mgL^{-1}$ BAP | 0.7519 e | 0.3695 bc | 1.3791 c | 1.2301 b |

**Note:** Dic= 4 $mgL^{-1}$Dicamba; Exp.= Explant; Means in a column followed by the same letter are not significantly different at 5% level of LSD

## 3. Result and Discussion

At 4 weeks in culture, calli were formed on all tested media. However, the rate of callus growth varied between 4 mgL-1 Dicamba in single plant growth regulator as a control and in combination with different concentrations of BAP tested. All treatment affected on all parameters observed either on leaf or petiole explants. In all treatments, the percentage of callus formation and fresh weight seemed to increase on 4 mgL-1 Dicamba combined with BAP compared with on media containing only 4 mgL-1 Dicamba, even the increase of BAP concentration tended to increase of fresh weight (Fig. 1 and Table 1). This result confirmed the statement of [19] that most of dycotyledonous plants required both auxins and cytokinins in growth medium for callus initiation. This fresh weight was useful if the callus would be regenerated become plantlet or be produced for secondary metabolite because plantlet and secondary metabolite production would increase.

In this research media of culture could influence fresh weight of callus produced. Statistical analysis proved that there were significantly different among media used on fresh weight of callus derived from leaf explant and from petiole explant both at induction and at proliferation stages. In general, media containing only 4 $mgL^{-1}$ Dicamba gave the lowest fresh weigth, on the contrary if in combination with 1.1 mgL-1 BAP gave the highest fresh weight of callus derived from leaf explant, while in combination with 0.9 mgL-1 gave the highest fresh weight of callus derived from petiole explant (Table 1). The effect of cytokinins is most noticeable in tissue cultures where they are used, often together with auxins, to stimulate cell division and control morphogenesis [23]. Meanwhile subculturing often becomes imperative when the density of cells is needed to increase the volume of a culture [24]. Subculturing plays a vital role in accumulation of secondary metabolites. This is proved from result of research on *Centella asiatica* (L.) in which the highest asiacotide content was detected in the first subculture of callus [11].

Figure 1 showed that the overall growth rate of callus on the media treatment enriched with BAP was better than on media containing Dicamba only, especially on explants derived from petiole. While the growth rate of callus derived from petiole explants was mostly higher than that of from leaf explants, this took place on all media either in callus induction or in callus proliferation. This result was opposite with the result on this plant from [12], [20],[21] that leaf explants are reported to be the best explant for callus induction compared to the other parts. The growth rate of callus derived from leaf explants on induction medium appeared slightly or somewhat varied with increasing concentrations of BAP. When

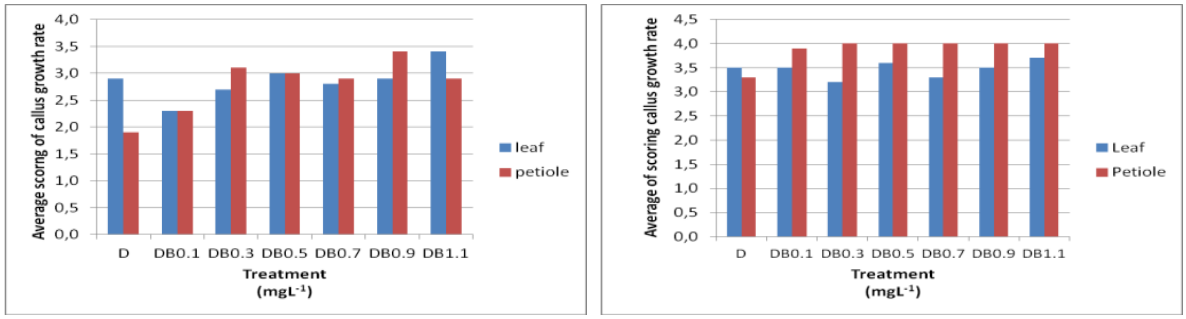
compared to the growth rate of callus derived from leaf explants on induction medium then only on media supplemented with 0.5 and 1.1 mgl-1 BAP seemed better than on media containing only 4 mgl-1 Dicamba.

In general, the research result showed that combination of auxin (Dicamba) and cytokinin (BAP) gave better callus growth than only auxin (Dicamba). This result was similar to the results from [21], [22] that 2,4-D (auxin) and BAP (cytokinin) could increase the callus growth.

Cytokinin's positive role in callus formation is thus likely to be on the subsequent periclinal divisions which are supported by the analysis of auxin and cytokinin activity during callus formation. This is a reason why the growth rate and fresh weight of callus on media containing Dicamba in combination with BAP was better than those on media containing Dicamba only. While the increasing of growth rate and fresh weight of callus at proliferation stage was caused by auxin activity that was high during the initial stages, but then decreased as cytokinin activity cocomitantly increased in the dividing callus tissue [18].

From this research result, it was known that response of the explants to the morphology of callus (texture and color) was different dependent upon the concentration of cytokinins. It means that the different types of explants produced the different callus morphology if they were cultured on media containing different plant growth regulators and concentrations. This result was confirmed by [23] Zia et al. (2007) that callus morphology and induction response much depended on explant source, explant size, medium content and plant growth regulator in media and culture condition as well.

Morphology of callus in this research was very various both in texture and in color. Fig. 2 demontrates that in callus induction, callus morphology produced in the same treatment varried but most of callus derived from leaf explant were friable compact while callus derived from petiole were friable. In callus proliferation, most texture of the callus both derived from leaf and petiole was friable compact (Fig. 2). Hence, the differences of callus texture in callus induction compared with in callus proliferation were on the percentage of compact callus where higher percentage occured in proliferation. This could be caused by the accumulation of BAP content on media, because the texture of callus on media containing only 4 $mgL^{-1}$ Dicamba was still friable. This result showed that the addition of BAP on media could increase the precentage of compact callus formation. From the previous studies indicated that the callus texture could be used to generate the callus to be plantlet, however, such texture was not suitable for cell suspension to produce secondary metabolites because the texture of callus needed in cell suspension was the friable callus.




(A) (B)

Fig. 1. Average scoring of callus growth rate (A) at induction stage and (B) at proliferation stage

Table 2. Color of callus derived from leaf and petiole explants on induction and proliferation medium

| Treatment | Induction | | Proliferation | |
|---|---|---|---|---|
| | Leaf Exp. | Petiole Exp. | Leaf Exp. | Petiole Exp. |
| Dic | Cream | Cream | Brownish cream | Brownish cream |
| Dic + 0.1 $mgL^{-1}$ BAP | Brownish cream | Whitish cream | Brownish cream | Brownish cream |
| Dic + 0.3 $mgL^{-1}$ BAP | Brownish cream | Whitish cream | Brownish cream | Brownish yellow cream |
| Dic + 0.5 $mgL^{-1}$ BAP | Greenish cream | Brownish cream | Brownish cream | Brownish yellow cream |
| Dic + 0.7 $mgL^{-1}$ BAP | Greenish cream | Brownish cream | Brownish yellow cream | Brownish yellow cream |
| Dic + 0.9 $mgL^{-1}$ BAP | Whitish cream | Brownish cream | Brownish yellow cream | Brownish yellow cream |
| Dic + 1.1 $mgL^{-1}$ BAP | Whitish cream | Whitish cream | Brownish yellow cream | Brownish yellow cream |

***Note:*** *Dic= 4 $mgL^{-1}$Dicamba; Exp.= Explant*

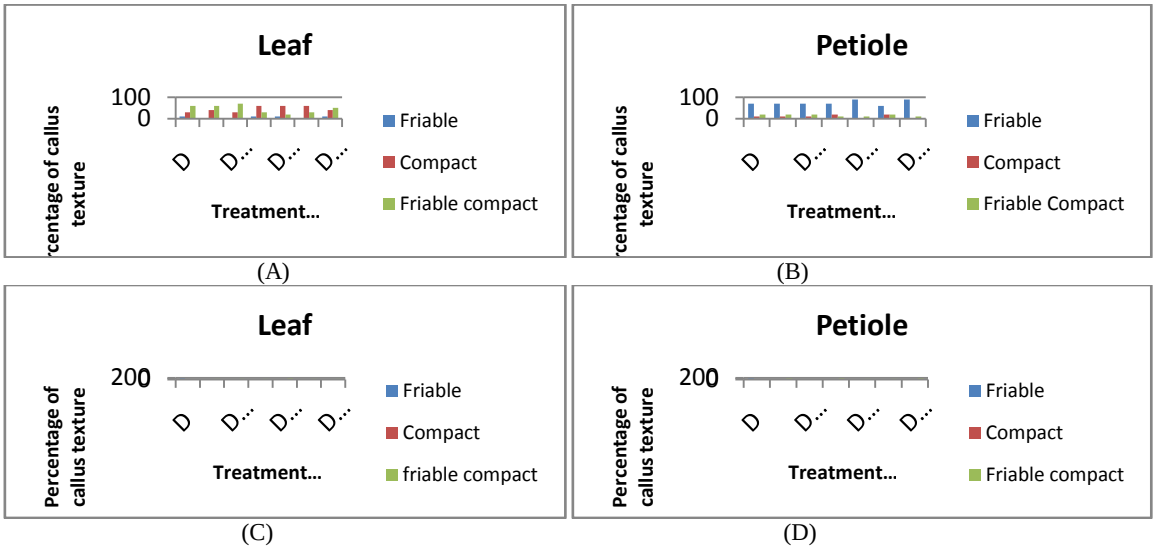



Fig. 2. Morphology of callus based on friable and compact texture. Above: derived from leaf explant (A) and petiole explant (B) in callus induction, 4 weeks of culture; Below: derived leaf explant (C) and petiole explant (D) in callus proliferation, 8 weeks of culture.

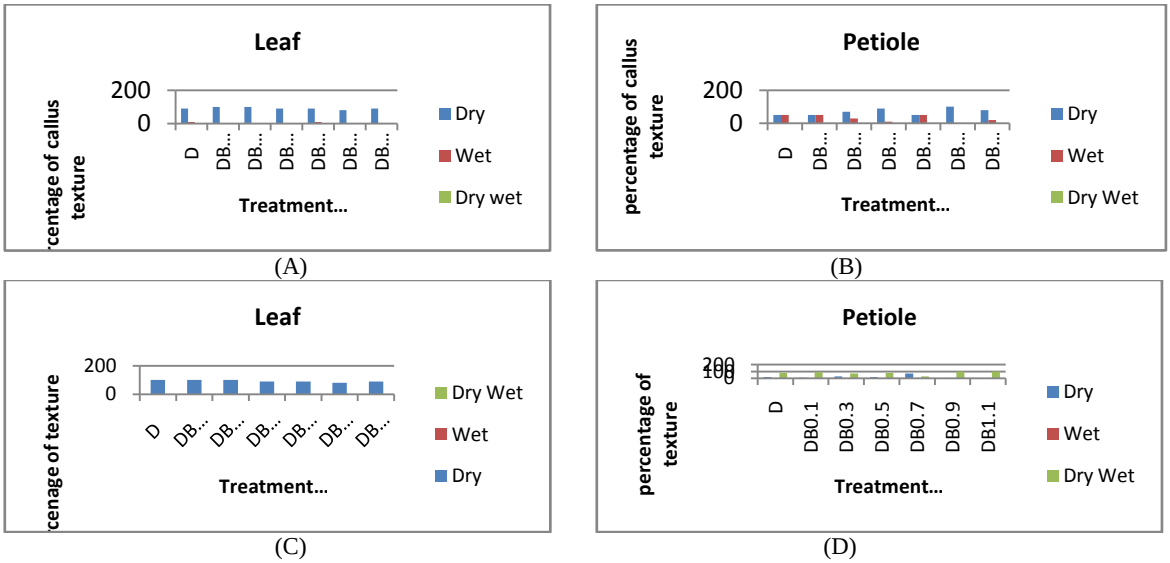
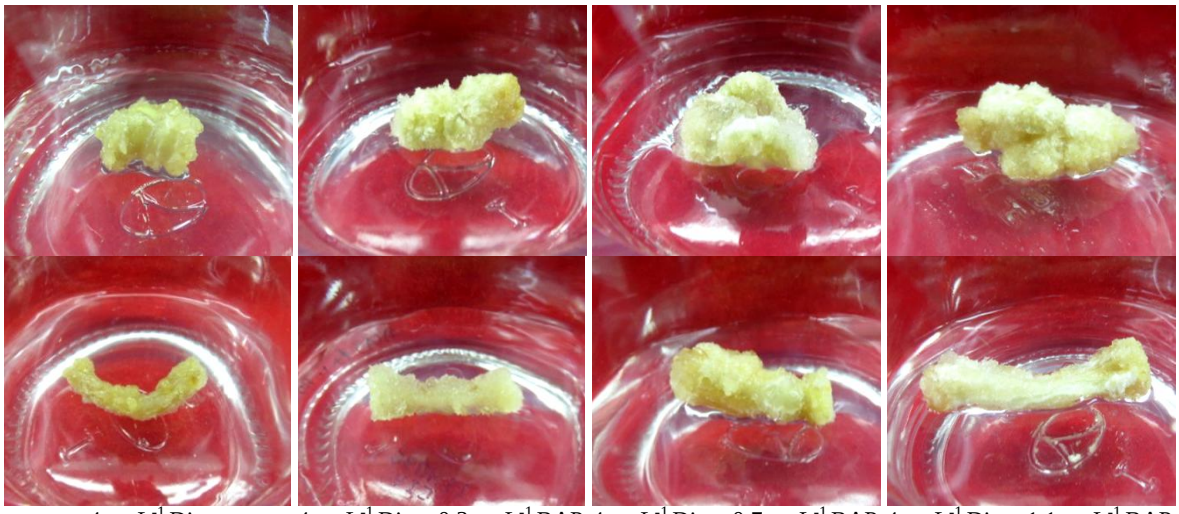
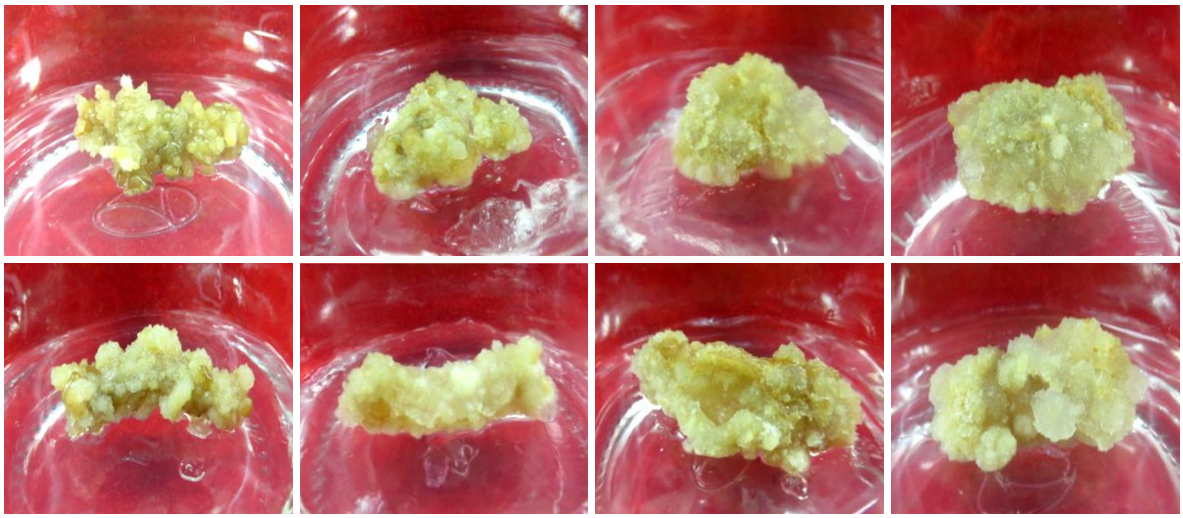


Fig. 3. Morphology of callus based on dry and wet texture. Above: derived from leaf explant (A) and petiole explant (B) in callus induction, 4 weeks of culture Below: derived leaf explant (C) and petiole explant (D) in callus proliferation, 8 weeks of culture.



4 $mgL^{-1}$ Dic | 4 $mgL^{-1}$ Dic + 0.3 $mgL^{-1}$ BAP | 4 $mgL^{-1}$ Dic + 0.7 $mgL^{-1}$ BAP | 4 $mgL^{-1}$ Dic + 1.1 $mgL^{-1}$ BAP

**Fig. 4.** Calus derived from leaf explant (above) and petiole (below) on induction medium, 4 weeks of culture



4 $mgL^{-1}$ Dic | 4 $mgL^{-1}$ Dic + 0.3 $mgL^{-1}$ BAP | 4 $mgL^{-1}$ Dic + 0.7 $mgL^{-1}$ BAP | 4 $mgL^{-1}$ Dic + 1.1 $mgL^{-1}$ BAP

**Fig. 5.** Calus derived from leaf explant (above) and petiole (below) on proliferation medium, 8 weeks of culture

Color of callus at the fourth week varied such as cream, brownish cream, greenish cream dan whitish cream (Tabel 2). In previous study by using various types of auxins the callus were easy to browning if not soon subcultured to fresh medium after the fourth week of culture [17]. Therefore, in this research BAP at various concentrations were added into media. Most of callus subcultured on the same media formulation as at induction stage yielded brownish yellow cream callus (Tabel 2). This might be caused by the growth of plant material in a closed vessel eventually leads to the accumulation of toxic metabolites

and the exhaustion of the medium [18]. Subsequently Consequently, most of the callus color at the proliferation stage was brownish.

The green callus was obtained from media of combination between 9.88 µM BAP + 1.08 µM NAA in centella [24], while light green callus was obtained from media enriched with 2,4-D and BAP, and greenish yellow callus was obtained from media with NAA and BAP [25]. The result of this research was supported by [12] who stated that *in vitro* technique may help in the conservation of species and possibly lead to the sysnthesis and extraction of active compound from callus culture sources.

It appreared that callus of centella in this research was easy to brown as the results of several previous researches. The media containing 2,4-D + BAP or Kinetin promoted rapidly callus growth from both leaf and stem explants which subsequently turned brown whitin 4 weeks of culture [26]. Accordingly [27] that 1 $mgL^{-1}$ auxins or wihout any plant regulator caused the callus began to turn brown after two weeks of culture. This phenomenon could be a base reason to looking for optimatization media to get callus that not easy to brown in the future. Cytokinin activity is a key element in establishing the organization and regulating cell division with genetic studies indicating that cytokinin is a positive regulator of cell proliferation [18].

## Conclusion

The best media formulation for induction and proferation of callus derived from leaf explant was 4 mg/L Dicamba combined with 1.1 mg/L BAP, which was indicated by the highest percentage of callus formation (100%) and fresh weight of calli i.e. 0.75 g and 1.38 g, respectively at the induction and proliferation stages; whislt for induction and proferation of callus derived from petiole explant was 4 mg/L Dicamba combined with 0.9 mg/L BAP in which the fresh weight of calli was 0.48 g and 1.58 g, respectively at the induction and proliferation stages. In addition the texture of callus produced from 4 mg/L Dicamba combined with 1.1 mg/L BAP was friable at the induction stage and friable compact at the proliferation stage, while that from 4 mg/L Dicamba combined with 0.9 mg/L BAP was friable at the induction stage and friable compact at the proliferation stage.

## Acknowledgments

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## References

[1] M. Bonfill, S.Mangas, R.M. Cusidó, L. Osuna, M.T. Pinol, J. Palazón J. 2006. Identification of triterpenoid compounds of *Centella asiatica* by thin-layer chromatography and mass Spectrometry. Biomed Chromatogr. 20:151–153.

[2] A.H. Siddiqui, S.T. Ali, S. Khan, S, Begum. 2006. Chemical constituent of *Centella asiatica*. Journal of Asian Natural Products Research 9(4): 407-414.

[3] P. Hashim. 2011. Mini review: *Centella asiatica* in food and beverage applications and its potential antioxidant and neuroprotective effect. International Food Research Journal 18(4): 1215-1222.

[4] V. Seevaratnam, P. Banumathi, M.R. Premalatha, S.P. Sundaram, and T. Arumugam. 2012. Functional properties of *Centella asiatica* (L.): A review. International Journal of Pharmacy and Pharmaceutical Sciences 4(5): 8-14.

[5] K. Joshi, P. Chaturvedi. 2013. Therapeutic efficiency of efficiency of *Centella asiatica* (L.) Urban, an Underutilized green leafy vegetable: An overview. International Journal of Pharma and Bio sciences 4(1): 135-149.

[6] G. Sivakumar, S. Alagumanian, and M.V. Rao. 2006. High frequency *in vitro* multiplication of *Centella asiatica*: an important industrial medicinal herb. Engineering of Life Science 6(6): 597-601.

[7] D.K. Mahapatra, B. Kumar. 2012. Ancient and pharmacological review on *Centella asiatica* (mandukparni): A potential herbal panacea. International Journal of Research and Reviews in

Pharmacy and Applied Science 2(6): 1062 – 1072.

[8] G. Subha, B. Ranjit. 2014. *Centella asiatica* is a potential indigenous herb of medicinal implication in ayuverda and clinical theraphy: a review. International Journal of Research in Pharmacy anf Life Science 2(1): 165-166.

[9] S. Nath, A.K. Buragohain. 2003. *In vitro* method for propagation of *Centella asiatica* (L.) Urban by Shoot tip Culture. J Plant Biochem Biotechnol 12: 167-169.

[10] K.P. Martin. 2004. Plant regeneraton through somatic embryogenesis in medicinally important *Centella asiatica* L. *In vitro* Cell Dev. Biolc. Plant 40(1): 586-591.

[11] K. Joshi, P, Chaturvedi, Subhpriya. 2013. Efficient *in vitro* regeneration protocol of *Centella asiatica* (L.) Urban: An endemic and underutilized butraceutical herb. African Journal of Biotechnology 12(33): 5164-5172.

[12] A. Patra, B. Rai, G.R. Rout, P. Das. 1998. Successful plant regeneration from callus cultures of *Centella asiatica* (Linn.) Urban. Plant Growth Regulation 24: 13-16.

[13] M. Ikeuchi, K. Sugimoto, A. Iwase. 2013. Plant callus: Mechanisms of induction and repression. Review. Plant Cell 25: 3159-3173.

[14] R.L.M. Pierik. 1987. *In vitro Culture of Higher Plant*. Martinus Nijhoff Publisher, Dordrecht 321p.

[15] H.N. Sholapur, F.S. Dasankoppa. 2011. Effect of subculturing and phytohormones on accumulation of asiacotide in callus cultures of *Centella asiatica* (L.) Urban. Indian Journal of Novel, Drug Delivery 3(2): 149-153.

[16] K. Ruslan, A. D. Selfitri, S.A. Bulan, Y. Rukayadi, Elfahmi. 2012. Effect of *Agrobacterium rhizogenes* and elicitation on the asiacotide production in cell cultures of *Centella asiatica*. Pharmacology Magazine 8(3):111-115.

[17] S. Rahayu, I. Roostika. N. Bermawie. 2016. The effect of types and concentrations of auxins on callus induction of *Centella asiatica*. Journal of Nusantara Biosains (Inpress).

[18] G.E. Schaller, A. Bishopp and J.J. Kieber. 2015. Review. The Yin-Yang of hormones: Cytokinin and auxin interactions in plant development. The Plant Cell Preview, www.aspb.org. American Society of Plant Biologist p. 1-20.

[19] E.F. George, P.D. Sherrington. 1984. *Plant Propagation by Tissue culture*. Exergetics limied, England.

[20] S. Nath, A.K. Burogahain. 2005. Establishment of callus and cell suspension cultures of *Centella asiatica*. Biologia Plantarum 49(3): 411 – 413.

[21] S. Gandhi, A. Giri. 2013. Production and quantification of Asiatic acid from in vitro raised shoots and callus cultures of *Centella asiatica* (L.) Urban. Ann. Phytomed., 2(1): 95-101.

[22] S. Mercy, N. Sangeetha, D. Ganish. 2012. *In vitro* production of adventitious roots containing asiacotide from leaf tissue of *Centella asiatica* L. In vitro Cell Development and. Biology Plant 48:200-207.

[23] E.F. George 2008. Plant Propagation by Tissue Culture. 3rd ed. Springer, Berlin.

[24] A. Varhney, M. Anis. 2014. *Trees: Propagation and Conservation*. Springer India p. 11- 25.

[25] Y. Bibi, M. Zia, S. Nisa, D. Habib, A. Waheed. F. M. Chaudahary. 2011. Regeneration of Centella asiatica plants from non-embryogenic cell lines and evalution of antibacterial and antifungal properties of regenerated calli and plants. Journal of Biological Engineering 5 (13):2-8.

[26] A.R. Arekar, S.S. Barve. 2005. *In vitro* regeneration of *Centella asiatica*, Linn. Journal of Cell and Tissuee Research 5(2): 435-438.

[27] S. Rao, K. Usha, Arjun. 2015. Production of secondary metaboliteees from callus cultures of *Centella asiatica* (L.) Urban. Annals of Phytomedicine 4(1):74-78.

[28]

# Triglyceride lowering effect of *Garcinia atroviridis* leaf tea from Sijunjung - West Sumatra on obese subjects in Medan, North Sumatra

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## Abstract

The worldwide prevalence of obesity increased more than doubled between 1980 and 2014. Overweight, obesity, and related non communicable diseases, are largely preventable. Supportive environments and communities are fundamental in shaping people's choices, by making the choice of healthier foods and regular physical activity the easiest choice,to prevent overweight and obesity.The food industry, including household industries, can contribute by ensuring healthy- nutritious choices are available to consumers. The household industries in Lubuk Tarok District of Sijunjung West Sumatra produces tea of GA leaves packed in tea bags, people drinking GA-tea to reduce cholesterol levels and waist circumference. Hydroxycitric acid (HCA) is the principal acid of the *Garcinia atroviridis* (GA) or asam glugur fruit rinds. HCA is a potent inhibitor of ATP-citrate lyase. The inhibition of this enzyme limits the availability of acetyl-CoA units required for fatty acid (FA) synthesis and lipogenesis. Animal studies indicated that HCA suppresses the FA synthesis, lypogenesis, and induced weight loss. The aim of this study was to determine the triglyceride levels lowering effect of GA-tea in obese subjects compare with control. Participants in this study were obese male and female adults, who were divided into GA-tea group (T-group) and control (C-group). All participants performed walking activity as conducted by investigator. Anthropometric measurement and laboratory test were performed pre- and post-treatment. In the results, thedecline change of triglyceridelevels was found in both GA-tea and control group. It was concluded that the lowering change of triglyceride levels was not significantly different between both groups.

Keywords: triglyceride levels, obese, Garcinia atroviridis

## 1. Introduction

Obesity and overweight are defined as abnormal or excessive fat accumulation that may impair health. The worldwide prevalence of obesity more than doubled between 1980 and 2014. World Health Organization (WHO) estimated that 39% of adults aged 18 years and over were overweight in 2014, and 13% were obese. Most of the world's population live in countries where overweight and obesity kills more people than underweight. [1] The fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended. [1]

Raised BMI is a major risk factor for noncommunicable diseases (NCDs) such as; cardiovascular diseases, diabetes, musculoskeletal disorders, and some cancers. The risk for these NCDs increases with increases in body mass index

(BMI).[1]Overweight and obesity, and the related NCDs, are largely preventable. Supportive environments and communities are fundamental in shaping people's choices, by making the choice of healthier foods and regular physical activity the easiest choice to prevent overweight and obesity.[1]The "*WHO Global Strategy on Diet, Physical Activity and Health*" calls upon all stakeholders to take action at global, regional and local levels to improve diets and physical activity patterns at the population level.[1]

At the individual level, people can limit energy intake and engage in regular physical activity (60 minutes a day for children and 150 minutes spread through the week for adults.[1]At the societal level it is important to support individualsthrough sustained implementation of evidence based and population based policies that make regular physical activity and healthier dietary choices available, affordable and easily accessible to everyone. The food industry can play a significant role in promoting healthy diets by ensuring that healthy and nutritious choices are available and affordable to all consumers, ensuring the availability of healthy food choices and supporting regular physical activity practice in the workplace. [1]

*Garcinia atroviridis*,known as asam gelugur grows to a height of 20 m and has long trunk, smoooth grey bark and drooping branches. The leaves are dark green, shiny, long narrow with a pointed tip and upturned edges. [2]. The ripe fruits of *G.atroviridis* which are bright orange-yellow used by Indonesian native as a flavoring ingredients in Indonesia cuisine, and to give acidity to cooked dishes in place of tamarind. [2]. The fruit of *G.atroviridis* contains fruiting acids such as citric acid, tartaric acid, malic acid and ascorbic acid. The principal acid of the fruit rinds of *G.atroviridis* ishydroxycitric acid (HCA, or (-)-HCA).[2]Hydroxycitric acid is found in the fruits and in the rind of certain Garcinia fruits [3].

ATP-citrate lyase (ACL) is one of the three citrate enzymes, which catalyze the same bond-making and -breaking reaction which involves the equilibrium of citrate with oxalacetate and an acetyl moiety. [4] ATP citrate lyase, a cytosolic enzyme, cleaves citrate so that acetyl CoA may be used for fatty acid and other biosyntheses. ATP Citrate (pro-3S) -Lyase is an enzyme that, in the presence of ATP and CoenzymeA, catalyzes the cleavage of citrate to yield acetyl CoA, oxaloacetate, ADP, and orthophosphate. This reaction represents an important step in FA biosynthesis. [4].

Hydroxycitric acid was shown to be a potent inhibitor of ATP citrate lyase, which catalyses the extramitochondrial cleavage of citrate to oxaloacetate and acetyl-CoA: citrate+ATP+CoA→ acetyl-CoA + ADP+Pi+ oxaloacetate. The inhibition of this reaction limits the availability of acetyl-CoA units required for fatty acid (FA) synthesis and lipogenesis during a lipogenic diet, that is, a diet high in carbohydrate. Animal studies indicated that (-)-HCA suppresses the FA synthesis, lypogenesis, food intake, and induced weight loss. In vitro studies revealed the inhibition of FA synthesis and lipogenesis from various precursors [3].

## 2. Material and Methods

### *2.1. Participants*

This study was designed as a case-control study to compare the TG levels lowering effect of *G.atroviridis* leaf tea between T- group and C-group.

The study was conducted on a sample of 30 participants at Medan, North-Sumatra between February 2016 and June 2016.

The criterion for participants recruitmentin the present study were adult, male and female, with obesity. The diagnostic criteria of obesity and waist circumference used in the present study were according to The International Obesity Taskforce proposed classification of BMI categories for Asia.

Obese was classified for subject with BMI ≥25 kg/$m^2$, with WC≥ 80 cm for women and ≥ 90 cm for men.[5],[6]All anthropometric were made with standard techniques: body weight was measured with digital scales to within 0.1 kg, with arms on side, legs straight, knees together, and without heavy clothing; body height was measured barefoot by stadiometer to within 0.5 cm; circumferences to within 1mm with stretch resistant plastic tapes, with waist mid-way between the lowest rib and the iliac crest with the subject standing at the end of gentle expiration. [7],[8]

The exclusion criteria were a history of heart disease, diabetes, use of lipid-lowering therapy, use of drugs affecting insulin resistance, oral hypoglycemic pills or insulin, pregnancy. All participants gave written informed consent.

The pre-treatment physical examination performed were measurement for height, weight, andWC. Blood samples were drawn for measurements of totalcholesterol, LDL-cholesterol, HDL-cholesterol, non-HDL cholesterol, TG, and fasting blood glucose (FBG). The laboratory test was performed after an overnight 12 hour fast. The post-treatment measurement were performed after completing 4-weeks treatment. Serum lipid profile test done in fasting blood specimen. Fasting refers to 12 overnight complete dietary restriction with the exception of water and medication, due to the reason that post prandial TG remain elevated for several hours [9].

Selected thirty participants (15 men, 15 women) were divided into two group by randomization; control group (15 subjects) and Garcinia Tea group (15 subject). Randomization was performed by random number generation, and group assignment was placed in a sealed envelop.

The primary endpoint of this study was to compare the lowering effect of *G.atroviridis leaf tea* on TG levelsof obese subjects after 4 weeks of tea consumptionagainst control. The secondary endpoints were body indices (body weight, body height to measure BMI), waist circumferences (WC), and laboratory values.

The study were approved by Ethical Committee of Medical Faculty of University of Andalas, Indonesia and conducted according to the Declaration of Helsinki.

*2.2. Procedure*

One sachet of *G.atroviridis* leaf tea brewed into 200 ml of hot water (80$^o$C) and allowed until decreased in tempreature, this tea could be drink immediately or any time all day long by participants in tea group.

All of participants attended walking activity three times a week, 45 minutes each time, for 4 weeks. Walk duration was measured by stopwatch. Walk distance was also measured in each activity.

*2.3. Material*

Fresh blood samples were analyzed to measure plasma levels of fasting plasma glucose, total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol.

*2.4. Statistical Analysis*

For characteristic data not normally distributed, results are given as medians (with min-max). For data normally distributed, results are given as mean ± SD.

Mann-Whitney U test was employed in data analyses.

## 3. Result and Discussion

The clinical characteristics of subjects are shown in Table 1 (Pre-treatment characterstic) and Table 2 (Post- treatment characteristic).

Pre-treatment measurement for BMI ranged from 26.83 - 42.93 kg/$m^2$ for control group, and 25.63 -36.61 kg/$m^2$ for Tea group. Pre-treatment serum triglyceride levels ranged from 79 - 550 mg/dl for control group and 74-843 for Tea group.

Table 1. Baseline (pre-treatment) characteristics

| | Control | Tea |
|---|---|---|
| N | 15 | 15 |
| Age, y | 47.7 (30.9 - 59.1) | 50.3 (31.4 - 58.3) |
| BMI, kg/m$^2$ | 30.89 (26.83 - 42.93) | 31.97 (25.63 - 36.61) |
| WC, cm | 107.00 (90 - 112) | 107.00 (94 - 114) |
| FBG, mg/dl | 103.00 (78 -171) | 116.00 (84 - 581) |
| TG, mg/dl | 179.00 (79 - 550) | 175.00 (74 - 843) |

Table 2. Post-treatment characteristics

| | Control | Tea |
|---|---|---|
| BMI, kg/m$^2$ | 30.61 (25.80 - 42.60) | 31.39 (25.07 - 35.54) |
| WC, cm | 104.00 (88 - 111) | 105.00 (92-113) |
| FBG, mg/dl | 85.00 (73 -139) | 89.00 (79 - 268) |
| TG, mg/dl | 136.00 (74 - 331) | 119.00 (66 - 367) |

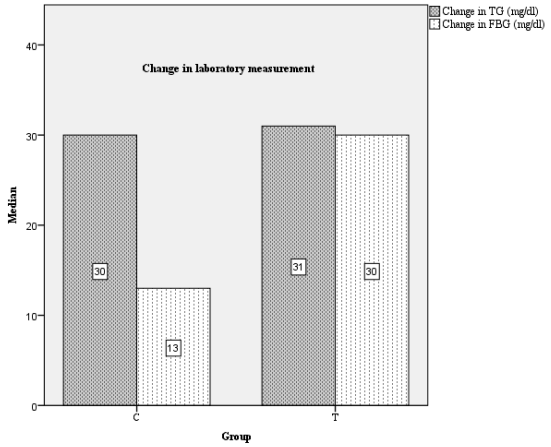


Figure1. Change in Laboratory measurement (serum TG level (mg/dl), FBG (mg/dl))

Table 2 contain the declining change of WC, BMI (Table 1 and Table 2), and the laboratory indices (FBG levels, and TG levels) after completing the 4 weeks treatment periods, both in in control and Garcinia Tea Group.

The alteration or lowering change of TG levels in the control group and Garcinia Tea group presented as median (min-max); 30.00 (-96 - 219) and 31,00 ( -147 - 617) consecutively.

Mann-Whitney U test performed and resulted with with*p*value 0.72 (*p*> 0.05), meansstatistically no significant difference of the TGlevel change in the tea group versus control, hence the null hypothesis was retained.The median of lowering change of the TG levels were different between groups, while mean rank of control group and tea group were 14.93 and 16.07 consecutively, it suggested that the tendency of changing in TG level in the tea group was greater than in the control group.

Total walking track (twelve activities) of the Control and Tea Group (mean ± SD) were (60.873± 6.591) and (60.200± 4.8971), consecutively, while average walking track of the Control and Tea Group during treatment periode were (3.8046 ± 0.41193) and (3.7625 ± 0.30607), respectively. Statistically, mean difference of total walking track between groups was not different significantly (mean difference 0.673, p 0.753, 95% CI (-3.6695 - 5.0612). Also, mean difference of average walking track was not significantly different between groups (mean difference 0.04208, p 0.753, and 95%CI (-0.22934 - 0.31351).

*3.1. Obesity and Triglyceride Levels*

Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health. Body mass index is a simple index of weight-for-height that is commonly used to classify overweight and obesity in adults. BMI is defined as a person's weight in kilograms divided by the square of body height in meters (kg/m$^2$). [1]

The background focusing on triglyceride in this study was due to a cross-sectional study conducted among adults and elderly people in Bambuí, Brazil found that 14% of obesity associated with high/very high TG and arterial hypertension. Diabetes was found in 3.7% and was associated with large WC, excessive saturated fatty acids and arterial hypertension. Triglyceride and obesity were the factors associated with chronic NCDs, others were obesity, high total

cholesterol, and excessive consumption of saturated fatty acids.[10]

Metabolic syndrome was present 15.6% of subjects who had a lower insulin sensitivity index (*S*i ), higher intra- abdominal fat (IAF) and subcutaneous fat (SCF) areas compared with subjects without the syndrome ($P < 0.001$). Multivariate models including *S*i, IAF, and SCF demonstrated that each parameter was associated with the syndrome. Intra-abdominal fat was independently associated with all five of the metabolic syndrome criteria. In multivariable models containing the criteria as covariates, WC and TG levels were independently associated with *S*i and IAF and SCF areas ($P < 0.001$). Although insulin resistance and central body fat are both associated with the metabolic syndrome, IAF is independently associated with all of the criteria, suggesting that it may have a pathophysiological role. Of the NCEP criteria, WC and TG may best identify insulin resistance and visceral adiposity in individuals with a fasting plasma glucose <6.4 mmol/l [11].

Intra abdominal visceral fat, which is mainly composed of omental and mesenteric fat, may play a more important pathogenetic role or better reflect an underlying metabolic disorder than subcutaneous fat in the development of diabetes mellitus or hyperlipidemia. Disturbances in glucose and lipid metabolism are considered greater in visceral type than in subcutaneous type [12].Visceral fat accumulation is a feasible condition for the development of various human disease. Visceral fat tissue produces and secretes various biologically active molecules. FFAs secreted from visceral fat up-regulates hepatic genes for lipoprotein synthesis and are possibly related to hyperlipidemia. Adipocytokines produced by accumulated in visceral fat may be a causative factor in the development of various feature in visceral fat syndrome [13].

Plasma TG and low-density lipoprotein particle size predict subsequent coronary artery disease in Caucasian populations. Studies demonstrate the importance of TG levels as a risk factor for cardiovascular disease [14]. Prospective studies in consistently indicate moderate and highly significant associations between TG values and coronary heart disease risk. [15]South Asians compared with Europeans had a higher mean per cent body fat, lower insulin sensitivity, higher intramyocellular lipid content. In South Asians, the strongest associations of insulin sensitivity were with fasting plasma TG and waist : hip ratio (WHR) [16].

As mentioned above, at the societal and individual level, regular physical activity practice at workplace is one of the easiest choice to prevent obesity. Sustained implementation of evidence based and population based policies make thoese choices affordable. Physical activity combined with healthy dietary choice produced by local household food industry, are importants support for this effort. The example of physical activity with benefit is endurance exercise training is a physical activity [16]. Endurance exercise training results in decreased plasma free fatty acid turnover and oxidation during a 90- to -120 min bout of submaximal exercise because of a slower rate of FFA release from adipose tissue.[17]

### *3.2.* Hydroxycitric acid and *G.atroviridis*

Hydroxycitric acid is a derivative of citric acid and can be found in plant species native to South Asia such as *Garcinia cambogia*, *Garcinia indica*, and *Garcinia atroviridis*The findings indicate that *G. atroviridis* leaves and fruits have potential for use as a source of natural antioxidants and nutrients for therapeutic purposes against free radical mediated health conditions [18]. The fruit of *G.atroviridis* also contains fruiting acids such as citric acid, tartaric acid, malic

acid and ascorbic acid that have an antioxidant activity. The principal acid of the fruit rinds of *G.atroviridis* isHydroxycitric acid (or -(-)-HCA) [2]. Hydroxycitric acid is found in the fruits and in the rind of certain Garcinia fruits. [3]

*3.3. G.atroviridis dried leaves as the source of HCA.*

HCA source in the present study was packed as tea bag containing dried *G.atroviridis* leaves. Findings indicate that *G.atroviridis leaves and fruits* have potential use as a source of natural antioxidants and nutrients fot therapeutic purposes against free radical medeiated health conditions. *Garcinia atroviridis* leaves extracted at different temperatures have different antioxidant activities when assessed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. [19] As far as the authors know, there has been no research on the extraction temperature to obtain a certain level of HCA in one tea bag of *G.atroviridis*.

*3.4. Dosage and Effect of HCA or Garcinia extract.*

In vivo, (-)-hydroxycitrate is a highly effective inhibitor of FA synthesis by rat liver. Findings reported that ATP-citrate lyase is a lipogenic enzyme that catalyzes the critical reaction linking cellular glucose catabolism and lipogenesis, converting cytosolic citrate to acetyl-coenzyme A (CoA). Acetyl-CoA is further converted to malonyl-CoA, the essential precursor for FA biosynthesis. Liver - specific ACL abrogation markedly inhibited the expression of peroxisome proliferator-activated receptor gamma and the entire lipogenic program in the liver. Moreover, hepatic ACL deficiency resulted in significantly down-regulated expression of gluconeogenic genes in the liver as well as enhanced insulin sensitivity in the muscle, leading to substantially improved systemic glucose metabolism. Hence, targeted suppression of ACL in the liver, not only leads to protection against liver steatosis, but also to marked improvement in whole-body glucose metabolism. These findings establish direct physiological evidence and a crucial role of hepatic ACL in lipid and glucose metabolism[20],[21]. HCA was also demonstrated as a potent inhibitor of ATP citrate (pro-3S) lyase (EC 4.1.3.8) from rat brain [22].

In one of the previous studies, ingested of *G.cambogia* extract (provided by Nippon Shinyaku Co., Ltd., Kyoto, Japan) for 12 weeks. The active herbal was a 270-mg tablet containing 185.25 mg of *G cambogia* extract, three tablest to be taken 30 minutes before each meal, and the total daily dose was 1667.25 mg of *G cambogia* extract containing 1000 mg of HCA. The result of this sudy were; significantly reduced visceral, subcutaneous, and total fat areas compared with the placebo group (all indices $P<0.001$). Serum triacylglycerol values tended to decrease over time in the *G.cambogia* groupbut not significantly. It was concluded that *G.cambogia reduced abdominal fat accumulation in subjects, regardless of sex, with visceral fat accumulation type of obesity. Therefore,* G.cambogia was hypothesized may be useful for the prevention and reduction of accuulation of visceral fat [23].

The effects of HCA ingestion on trained or untrained subjects, or during rest and after exercise were also investigated in the following researches. In untrained woman, the effects of short-term HCA ingestion on endurance exercise performance and fat metabolism results with increased fat metabolism, which may be associated with a decrease in glycogen utilization during the same intensity exercise and enhanced exercise performance [24].

In untrained men, study on the effects of HCA ingestion on fat oxidation during moderate intensity exercise used 500 mg of HCA for 5 days combined with endurance exercise. The results suggested short-term

HCA ingestion increases fat oxidation in untrained men [25]. Although the treatment period in above study was shorter than our study (5 days vs 4 weeks), the uncertainty of the HCA concentration in Garcinia leaf tea may be a weakness of our study [25].

Acute effects of HCA supplementation on substrate utilization at rest and during exercise in endurance - trained humans was also investigated. Subjectsingested 3.1 mL/kg body wt of an HCA solution (19 g/L) both at 45 min and 15 min before exercise, and at 30 and 60 min after the start of exercise. A large amount of HCA (a total of 18 ± 0.4 g HCA divided over 4 doses of 4.4 ± 0.1 g) to increase and maintain high plasma HCA concentration during exercise. Ingestion of HCA was followed with increased plasma HCA concentration; ingestion of one dose of above HCA solution at t=-45, and t=-15 resulted in an increase in plasma HCA concentration up to 16.6 mg/L during rest. Ingestion one dose of HCA after 30 and 60 min of exercise was followed with further up increase of plasma HCA concentration up to 82.0 ± 4.8 mg/L No significant differences in total fat and carbohyrate oxidation rates were observed between trials. In results, HCA, large quantities of HCA was not increase total fat oxidation in vivo in endurance-trained humans. [26]The amount of HCA ingested (18 ± 0.4 g) in the present study is 6-30 times higher than the doses applied in other study [26, 27]. In the pilot study, plasma HCA concentrations increased over time after ingestion of a single dose of HCA 4.4 g, with maximal values were attained after 60 - 90 min, after which the concentration decreased. This study [26] demonstrated that ingested HCA is absorbed in the gastrointestinal tract and enters the systemic circulation. Which is of primary importance if HCA is to reach the target cells and induce its suggested effect on skeletal muscle fuel selection.

Results of present study shows that obese subjects in T-group and C-group experienced a decrease in TG levels (Table 1, Tabel 2, Figure 1). Decreased of TG levels in T-group were slightly greater than in C-group (31 mg/dl vs 30 mg/dl), and Mann-Whitney U test resulted with *p* value 0.72 ($p>0.05$). Different from previous study, present study used a tea bag made from *G.atroviridis* leaf brewed in a 200 ml of water, and may be drunk at any time of the day, during four weeks treatment. Other differences were that the concentration of HCA in 200 ml of Garcinia tea and its bioavailability have never been investigated before., Although study results in humans were contradictory[28], further investigation on HCA from *G.atroviridis* from West-Sumatra still needed to be extended before HCA can be recommended as a promising weight control and lipid lowering agent.

## Conclusion

As far as this author knows, this is the first study and report demonstrating the the triglyceride lowering efect of *Garcinia atroviridis* leaf tea produced in Sijunjung District- West Sumatra on obese subjects. Lowering change in triglyceride levels was found amongobese subjects after tea ingestion, and the lowering change of triglyceride levels was not significantly different compared with control group. Further study needed to provide more evidence on this product.

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## References

[1] World Health Organization. Obesity and overweight [Internet]. 2016. Available from: http://www.who.int/mediacentre/factsheets/fs311/en/

[2] Dweck A. A review of Asam Gelugor (Garcinia

atroviridis) Griff.ex T.aders [Internet]. Dweck Data. Available from: http://www.dweckdata.com/research_files/garcinia_atroviridis.pdf

[3] B. S. Jena, G. K.Jayaprakasha , R. P. Singh, and K. K.Sakariah . "Chemistry and biochemistry of (-)-hydroxycitric acid from Garcinia," *J Agric Food Chem.* vol.50, pp.10–22, 2002.

[4] A. C. Sullivan, M. Singh, P. A. Srere, and J. P.Glusker . "Reactivity and Inhibitor Potential of Hydroxycitrate Isomers with Citrate Synthase , Citrate Lyase , and ATP Citrate Lyase,"*J Biol Chem.*,vol. 252(13), pp.7583–90, 1977.

[5] World Health Organization West Region, International Association For The Study of Obesity, International Obesity TaskForce. The Asia Pacific perspective : Redefing obesity and its treatment [Internet]. 2000. p. 1–56. Available from: www.wpro.who.int/nutrition/documents/docs/Redefiningobesity.pdf.

[6] R. C Weisell. "Body mass index as an indicator of obesity,". *Asia Pacific J Clin Nutr*., vol.11 Suppl., pp. S681-4,2002.

[7]. M. E. Lean , T. S. Han , and C. E. Morrison. "Waist circumference as a measure for indicating need for weight management,". *BMJ*., vol.311, pp. 158–61, 1995.

[8]. S. M. Grundy, I. J. Neeland, Turer A. T. , and Vega G. L. "Waist circumference as measure of abdominal fat compartments,". *J Obes*., vol. 2013, pp.1-9, 2013.

[9]. H. Campos, C. Khoo, and F. M. Sacks,"Diurnal and acute patterns of postprandial apolipoprotein B-48 in VLDL, IDL, and LDL from normolipidemic humans,". *Atherosclerosis*., vol.181(2), pp. 345–51, 2005.

[10] A. C. S. Lopes, L. C. Santos, M. F. Lima-Costa, and W. T.Caiaffa, "Nutritional factors associated with chronic non-communicable diseases- the Bambuí Project: a population -based study,". *Cad Saúde Pública*., vol.27(6), pp. 185–91. 2011.

[11] D. B. Carr, K. M. Utzschneider, R. L. Hull, and K. Kodama,"Intra-abdominal fat is a major determinant of the National Cholesterol Education Program Adult Treatment Panel III criteria for the metabolic syndrome,". *Diabetes*, vol.53, pp. 2087–94. 2004.

[12] S. Fujioka, Y. Matsuzawa, K. Tokunaga, and S. Tarui "Contribution of intraabdominal fat accumulation to the impairment of glucose and lipid metabolism in human obesity,". *Metabolism*, vol.36(1), pp.54–9. 1987.

[13] T. Funahashi, T. Nakamura, I. Shimomura, and K. Maeda, "Role of adipocytokines on the pathogenesis of atherosclerosis in visceral obesity,". *Intern Med*., vol. 38(2), pp. 202–6, 1999.

[14] M. Austin, J. Hokanson, and K. Edwards. "Hypertriglyceridemia as a cardiovascular risk factor,". *Am J Cardiol*., vol.81(4A), pp.7B–12B,1998.

[15] N. Sarwar, J. Danesh, G. Eiriksdottir, and G. Sigurdsson, "Triglycerides and the risk of coronary heart disease,". 10 158 incident cases among 262 525 participants in 29 western prospective studies,"*Circulation*, vol. 115, pp. 450–8, 2007.

[16]. N. G. Forouhi, G. Jenkinson, E. L.Thomas , and S. Mullick, "Relation of triglyceride stores in skeletal muscle cells to central obesity and insulin sensitivity in European and South Asian men,". *Diabetologia*, vol.42, pp.932–5, 1999.

[17]. W. H. Martin, G. P. Dalsky, B. F. Hurley, and D. E.Matthews , "Effect of endurance training on plasma free fatty acid turnover and oxidation during exercise,". *Am J Physiol*., vol.28, pp. E708-714,1993.

[18] C. Roongpisuthipong, R. ,Kantawan,and W. Roongpisuthipong. "Reduction of adipose tissue and body weight : effect of water soluble calcium hydroxycitrate in Garcinia atroviridis on the short term treatment of obese women in Thailand,". *Asia Pac J Clin Nutr*., vol.16(1), pp. 25–9, 2007.

[19] I. Nursakinah, H. A. Zulkhairi, M. Norhafizah, and Md. B. Hasnah, "Nutritional content and in vitro antioxidant potential of garcinia atroviridis (asam gelugor) leaves and fruits,"*Malays J Nutr*., vol.18(3), pp. 363–71, 2012.

[20] Q. Wang, L. Jiang, S. Li, and Y. Yu, "Abrogation of hepatic ATP-citrate lyase protects against fatty liver and ameliorates hyperglycemia in leptin receptor-deficient mice,"*Hepatology*, vol.49, pp.1066–175, 2009.

[21] J. M. Lowenstein,"Effect of (-)-hydroxycitrate on fatty acid synthesis by rat liver in vivo,". *J Biol Chem*., vol. 246(3), pp.629–32, 1971.

22] A. Szutowicz, M. Stepien, W. Lysiak, and S. Angielski, "Effect of (-)hydroxycitrate on the activities of ATP citrate lyase and the enzymes of acetyl CoA metabolism in rat brain,"*Acta Biochim Pol*., vol.23(2–3), pp. 227–34, 1976.

[23] K. Hayamizu, Y. Ishii, I. Kaneko, and M Shen, "Effects of Garcinia cambogia ( Hydroxycitric Acid ) on Visceral Fat Accumulation : A Double-Blind , Randomized , Placebo-Controlled Trial,". *Curr Ther Res Clin Exp*., vol.64, pp.551–67, 2003.

[24] K. Lim, S. Ryu, H. S. Nho, and S. K. Choi, "(-)-Hydroxycitric Acid Ingestion Increases Fat Utilization During Exercise in Untrained Women,". *J Nutr Sci Vitaminol*., vol. 49(3),

pp.163–7, 2003.

[25] K. Tomita, Y. Okuhara, N. Shigematsu, and H. Suh, (-)-"Hydroxycitrate Ingestion Increases Fat Oxidation During Moderate Intensity Exercise in Untrained Men,". *BiosciBiotechnolBiochem*., vol. 67(9), pp. 1999–2001, 2003.

[26] L. J. C. Loon, J. J. M. Rooijen, B. Niese, and H. Verhagen, "Effects of acute ( - ) -hydroxycitrate supplementation on substrate metabolism at rest and during exercise in humans,". *Am J Clin Nutr*., vol. 72, pp. 1445–50, 2000.

[27] A. D. Kriketos, H. R. Thompson, H. Greene, and J. O. Hill,"(-)-Hydroxycitric acid does not affect energy expenditure and substrate oxidation in adult males in a post-absorptive state,". I*nt J Obes Relat Metab Disord*., vol.23(8), pp. 867–73, 1999.

[28] S. Heymsfield, D. Allison, J. R. Vasselli, and A. Pietrobelli, "Garcinia cambogia (Hydroxycitric Acid) as a Potential Antiobesity Agent,". *JAMA*, vol. 280(18), pp. 1596–600, Nov.1998.

# Preparation and Characterization of Ethanol Extract of Mychorryzae Induced Ginger as Raw Matherial for Anti Breastcancer Nanosuspension Formulation

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## Abstract

Ginger (*Zingiber officinale* Rosc.) one of the most widely used species of the ginger family, is a common condiment for various foods and beverages. It has been traditionally used in jamu formula related to its diseases treatments. Some pungent constituents present in ginger plants have potent antioxidant and anti-inflammatory activities, and some of them exhibit cancer preventive activity. The anticancer properties of ginger are attributed to the presence of chemical compound such as [6]-gingerol, [6]- shogaols, and zingerone. To increase the use of this plant in therapy, there has been done an integrated research. A research is started from investigation raw material in upstream part to study of bioactivity and product development in downstream part. This study presented development to obtain extracts and active fractions from mychorryzae induced ginger rhizome as raw material for anti-breast cancer nanosuspension formulation. Extraction was done by maceration with ethanol 96%, extract quality examination was conducted by the organoleptic checked, the determination of drying shrinkage, ash content, acid insoluble ash content, the levels of alcohol-insoluble material and content of water-insoluble materials. fractionation using solvents with different polarity such as n-hexane, ethyl acetate and methanol. The results showed that from 1 kg of fresh ginger rhizomes obtained ethanol extract as much (2.86% ± 0.49), viscous, brown color, distinctive smell of ginger. The ash content of the resulting extract is (18.15% ± 1.08), the levels of water-insoluble extract was (16.19% ± 5.84), acid insoluble ash content 0,14%±0,05, the levels of alcohol-insoluble material 15,94%±2,95 and content of water-insoluble materials 16,19%±5,84.

Keywords : Ginger; mychorryzae; anti breastcancer; 6-Gingerol; ethanol extracts

## INTRODUCTION

Ginger, the rhizome of *Zingiber officinale* Roscoe, one of zingiberaceae family is widely used around the world as spice for foods and baverages (Gupta *et al.,* 2008). This plant is also widely used as a traditional medicine globally (Ali *et al.,* 2008; Malekizadeh *et al.*, 2012). Ginger is one of herbs plants that growth well and widely spread in tropic and sub tropic region, include in Indonesia. Based on the morphology (size, shape, and color of the rhizome), in Indonesia there are three kinds of ginger, elephant ginger, emprit ginger and red ginger (Paimin and Murhananto, 1991).

This plant has received increased attention because of its antioxidant (El-Ghorab *et al.*, 2010; Mesomo *et al.*, 2012), antidiabetic (Afshari *et al.*, 2007), anti-inflammatory (Mighetti *et al.*, 2007) and anticancer activities (Shukla and Singh, 2007; *et al.,* 2011). Chemical contains of ginger are essensial oils and oleoresin. Oleoresin of ginger is non-volatile pungen component and

its major component have been identified as gingerol, paradol, shogaol, zingerone and resins (He, *et al.*, 1998; Ravindran, 2005; Hu *et al.*, 2011). When ginger was dried gingerol forming shogaol, which has a pungent odor and taste two times larger than gingerol. This is the background why dried ginger tasteless and odorless, sharper than the fresh ginger (McGee, 2004). Recent studies have focused on the potencies of Mychorrizae Arbuscular Fungi (MAF) to induced resistence of ginger wild desease and increased rhizome productivity (Suharti, *et al.*, 2011) the cytotoxic activity of MAF induced resistence ginger onto breast cancer (Suharti, *et al.*, 2014). To increase the use of this plant in therapy, there has been done an integrated research. A research is started from investigation raw material in upstream part to study of bioactivity and product development in downstream part. This study presented to obtain extracts and active fractions from mychorryzae induced ginger rhizome as raw material for anti-breast cancer nanosuspension formulation.

## MATERIAL AND METHODS

### Plant Materils and Sample Preparation

Fresh ginger purchased from the screen house of medicinal plant study center was washed cleand and chopped into sliced. Extraction was done by maceration with ethanol 96%, extract quality examination was conducted by the organoleptic checked, loss of drying determination, ash content, acid insoluble ash content, the levels of alcohol-insoluble material and content of water-insoluble materials. Fractionation using solvents with different polarity such as n-hexane, ethyl acetate and methanol. 1 kg of fresh ginger was put in a dark glass container and macerated with 1.2 liters of ethanol distillation for 3 days while occasionally stirring. Maceration performed three times. The extract separated by filtration. All extract Collected, then boiled with a vacuum evaporator to obtain a thick extract (FHI 1st ed. ,2008).

### Quality Inspection of Condensed Ginger Extract

Organoleptic examination

Organoleptic examination include shape, color, and odor.

Determination ofYield

The yield is calculated by weight of the resulting extract (g) divided by the weight of sample (g) multiplied by 100%.

Determination of Drying Shrinkage

Weigh carefully the 1 g crude extract in shallow weighing bottle with a lid that previously been heated at a temperature-setting (105 °) and weigh again. Flatten the material in the weighing bottle by shaking the bottle, up to a layer thickness of approximately 5 to 10 nm, enter the drying chamber, uncovered, to dry at a temperature of determination until the weight remains. Before each drying, leave the bottle in a closed state to cool in the desiccator (FHI 1st ed. ,2008).

Determination of ash content

A total of 1 g of substance which had been weighed, put into the crucible which has weighed and heated then leveled. Heat until exhausted charcoal, cooled and weighed. If charcoal wasn't be lost, add hot water and filtered at the same crucible. Enter the filtrate into the crucible, boiled, heated, then weighed. Calculate the ash content of the material that has dried up in the air (FHI 1st ed. ,2008).

Determination Acid Insoluble Ash Content

The ash obtained boiled in the determination of total ash with 25 mL of 2 N HCl for 5 minutes. Collect the parts that are not soluble in acid, filtered through ash-free

filter paper, wash with hot, heatedat the crucible until the weight remains. The ash content is not soluble in acid calculated to the weight of the test substances, expressed in% w / w.

Determination of material insoluble in alcohol

One g extract thick carefully into 250 ml erlenmeyer already containing 100 ml of alcohol, close the Erlenmeyer with a cork stopper by cotton, heat the mixture until boiling and then filtered by using a crucible flat-known weight of the paper filtration, dry filter paper that has been $105^0$ C residue in the oven for 1 hour, cooled in a desiccator for 30 minutes and weighed until the weight remains (SNI Gambir, 2000).

Determination of material insoluble in water

Condensed extract weighed as much as 1 g carefully into 250 ml Erlenmeyer already containing 100 ml of water. The mixture was heated to boiling and then filtered by using a flat crucible of known weight of the paper filtration, the filter paper that had contained the residue dried in an oven 105oC for 1 hour, cooled in a desiccator for 30 minutes and weighed until the weight remains (SNI Gambir, 2000).

Examination of Thin Layer Chromatography

TLC examination conducted to demonstrate the stain patterns that appear on the compound extraction by some corresponding mobile phase. The mobile phase used in this research is hexane-ethyl acetate (4: 1) used UV365nm UV254nm lights.

## RESULT AND DISCUSSION

Extract organoleptic examination includes the shape, smell and color. From the observation of the condensed extract of ginger is thick. While the color was tawny and a have a spesific odor. The organoleptic evaluation included one specific parameters by using the five senses and aim for the early introduction of a simple way.

Determining the yield of ginger extract of fresh rhizome sample, result of 2.86% ± 0.49. Results of analysis showed that the yield generated is greater, this may be due to the sample used was fresh not dried so that the compounds that are volatile dissolved during the maceration process takes place. Drying shrinkage obtained is 19.96% ± 4.50. Determination of drying shrinkage aims to show the number of compounds that evaporate or lost due to the heating of water and other volatile compounds.

The ash content obtained from the sample is 18.15% ± 1.08. Determination of ash content aims to provide an overview of inorganic contaminants such as mineral compounds contained in the extract. Insoluble extract concentration of alcohol is 15.94% 2.95. This indicates that the solubility properties of the extracts is more soluble in alcohol.

Levels of water-insoluble extract is 16.19% ± 5.84, this indicates that the second treatment is more soluble in water than the first and third treatment.

From examination of Thin Layer Chromatography showed that the pattern of stains that looked from the extracts. Chromatographic separation procedure is defined as solute by a dynamic differential migration process in a system consisting of two phases, one of which moves continuously in a certain direction and in which substances it showsdifferences in mobility due to differences in adsorption, partition, solubility, vapor pressure, the size of the molecule or ion charge density. (FHI 1st edition, 2008). Appellant applied that clove oil with Rf 0.65and gingerol with Rf 0.70 In the samples was determined by comparing the value of its Rf sample with Rf comparison.

**References**


Balachandran, S., Kentish, S. E. and Mawson, R. 2006. The Effects of Both Preparation Method and Season on the Supercritical Extraction of Ginger. *Sep. Purif. Technol.*, 48: 94–105.

Cheng, X. L., Liu, Q., Peng, Y. B., Qi, L. W. and Li, P. 2011. Steamed Ginger (*Zingiber officinale*): Changed Chemical Profile and Increased Anticancer Potential. *Food Chem.*,129: 1785–1792.

Departemen Kesehatan Republik Indonesia. 2008. *Farmakope Herbal Indonesia*.

Edisi I. Jakarta: Departemen Kesehatan Republik Indonesia.

El–Ghorab, A. H., Nauman, M., Anjum, F.M., Hussain, S. and Nadeem, M. 2010. A Comparative Study on Chemical Composition and Antioxidant Activity of Ginger (*Zingiber officinale*) and Cumin (*Cuminum cyminum*). *J. Agric. Food Chem*., 58: 8231–8237.

Hu, J. J., Guo, Z., Glasius, M., Kristensen, K., Xiao, L. T. and Xu, X. B. 2011. Pressurized Lliquid Extraction of Ginger (*Zingiber officinale* Roscoe) with Bioethanol: An Efficient and Sustainable Approach. *J. Chromatogr. A*., 1218: 5765–5773.

Malekizadeh, M., Moeini, M. M. and Ghazi,S. 2012. The Effects of Different Levels of Ginger (*Zingiber officinale* Rosc) and Turmeric (*Curcuma longa* Linn) Rhizomes Powder on Some Blood Metabolites and Production Performance Characteristics of Laying Hens. *J. Agr. Sci. Tech.*, 14: 127–134.

Mesomo, M. C., Scheer, A. P., Perez, E.,Ndiaye, P. M. and Corazza, M. L. 2012. Ginger (*Zingiber officinale* R.) Extracts Obtained Using Supercritical CO2 and Compressed Propane: Kinetics and Antioxidant Activity Evaluation. *J. Supercrit. Fluids*, 71: 102–109.

Ravindran, PN., Babu KN. 2005. Ginger the genus Zingiber. CRS Press. New York.

Shukla, Y., Singh, M. 2007. Cancer Preventive Properties of Ginger : A Brief Review. *J. Food Chem Toxicol*, 45 (5), 683-690.

Suharti, Habazar,T; Dachryanus; Nasir,N; Jamsari. 2011. Induksi Ketahanan Tanaman Jahe Terhadap Penyakit layu bakteri *Ralstonia solanacearum* ras 4 mengunakan Fungi Mikoriza Arbuskula. *Jurnal Hama Dan Panyakit Tropika* Vol 14 No 2.

Suharti, Dachriyanus, Wahyuni, S.F., 2014. Uji Aktivitas Sitotoksik Rimpang Tanaman Jahe yang diintroduksi Fungi MIkoriza Arbuskula Pada Sel Kanker Payudra T47D. Artikel Penelitian Hibah Bersaing (Unpublished).

Shukla, Y., Singh, M. 2007. Cancer Preventive Properties of Ginger : A Brief Review. *J. Food Chem Toxicol*, 45 (5), 683-690.

Supardjan, A.M. & Meiyanto, E., (2002). *Efek antiproliferatif pentagamavunon- 0 terhadap beberapa sel kanker*. Yogyakarta: Lembaga Penelitian Universitas Gadjah Mada.

Surh, Y. 1999. Molecular mechanism of chemopreventive effect of selected dietary and medicinal phenolic substances. J environ Pathol Toxicol Oncol. 428(1-2) : 305-327.

Tjay, T. H. & Raharja, K. 1991. *Obat-obat Penting: Khasiat, Penggunaan danEfek Sampingnya* (Edisi IV). Jakarta: PT Elex Media Komputindo.

Zakaria, F. R.dan Rajab, T. M. 1999. Pengaruh Ekstrak Jahe (Zingiber Officinale Roscoe) terhadap Produksi Radikal Bebas Makrofag Mencit Sebagai Indikator Imunostimulan Secara in-vitro. Persatuan Ahli Pangan Indonesia (PATPI). *Prosiding Seminar Naional Teknologi Pangan*, 707-716.

# Effect of Benzyladenine (BA) and Duration of Shading onGrowth and Quality of *Dracaena sanderiana and Codiaeum variegatum*

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## Abstract

This study was carried out to determine the effect of Benzyladenine (BA) and Duration of Shading on Growth and Quality of *Dracaena sanderiana* and *Codiaeum variegatum* during simulation of subsequent shipping conditions (in the dark room). The plants were sprayed with the cytokinins hormone at different consentrations 0 (control), 75, 150, 225 and 300 mg L-1. The data were analyzed using ANOVA followed with Duncan Multiple Range Test (DMRT). The Results showed that the growth and plant quality of *Dracaena sanderiana* and *Codiaeum variegatum* in terms of chlorophyll content, leaf drop, plant height and plant grade were significantly affected by BA. Application of BA at 225 mgL-1 under 6 weeks shading duration significantly increased chlorophyll content of Dracaena sanderiana. *Dracaena sanderiana* sprayed with BA at 225 mgL-1 and under 6 weeks shading duration had significant reduction in leaf drop. For *Dracaena sanderiana*, BA at 225 mgL-1 and shading duration 4 weeks resulted in higher of plant height compared to the control. The plant grade also improved with application of BA at 225 mgL-1. *Codiaeum variegatum* sprayed with BA at 150 mgL-1 before 4 weeks of shading duration reduced the percent leaf drop significantly. Application of BA at 150 mgL-1 decreased plant height increment with shading duration of 4 and 6 weeks. Application of BA at 150 mgL-1 and shading improved the plant grade.

**Keywords**: Dracaena sanderiana, Codiaeum variegatum, Benzyladenine, Shading, Quality, Dark room.

## 1. Introduction

Plants are classified into sun and shade plants depending on their adaptability to a selected light intensity [3]. The shade plants exhibited low dark respiration rates (0.06-0.16 µmole CO2 evolved dm-2 min-1) and low light compensation points. Shading decreased the light compensation point, indicating that light intensity requirement of the plants was reduced. Light intensity influences the amount of variegation in Dracaena sp. [3]. Leaf and quality losses due to dark storage durations of 4 to 12 days were less when *F. benjamina* were subsequently held under incandescent (INC) lamps compared to cool-white fluorescent (CWF) lamps [3].

In contrast, [3] found that *Codiaeum variegatum* (L) Blume displayed increased color intensity, as well as increased amount of variegation, under higher light levels. A curvilinear response to increase shade levels for total leaf area was found in *L. coccinea* Planch [3]; where the area increased with

each increment of shading from 0% to 63% but drastically declined under 92% shade.

The foliage plant industry is concerned with the quality retention of plants during production and subsequent indoor use. Most container-grown foliage plants used for interior decoration are sealed in boxes and shipped to the destination without light, resulting in zero net photosynthesis for several days. Acclimatization of intact plant, through production under condition of reduced light intensity prior to harvest, can greatly improved the retention of quality during and after storage [3].

During shipping, the plants receive no light and often experience extreme temperatures. Shipping stress can result in chlorosis of the lower leaves, bud malformation and abscission, and etiolated shoot growth [3]. [3] stored *F. benjamina* for periods of 0, 4, 8, or 12 days and found increasing leaf drop with periods of simulated shipping. However, Aphelandra and Philodendron tolerated simulated shipping in the dark for periods up to 9 days without reduction in quality except for Dieffenbachia [3]. Information on the required environmental conditions, adaptation and transportation effect of foliage plants such as *Dracaena sanderiana* and *Codiaeum variegatum* in Malaysia are lacking.

The objective of this experiment was to determine the effect of concentrations of BA and shading durations on postharvest quality of *Dracaena sanderiana* and *Codiaeum variegatum* and subsequently during simulated shipping condition.

## 2. MaterialandMethods

The study was conducted using a factorial experiment in a randomized complete block design (RCBD). This was a factorial experiment with different concentrations of BA, 0 (control), 75, 150, 225 and 300 mgL-1 and watering frequency, daily, every 4, 7 and 10 days. It was also a factorial experiment with two levels of BA and three (2, 4 and 6 weeks) durations of shading. The two levels of BA were 0 and 225 mgL-1 for *Dracaena sanderiana* and 0 and 150 mgL-1 for *Codiaeum variegatum*. All treated plants were placed in the shade (63%) houses at Malaysian Agriculture Research and Development Institute (MARDI).

Plants were watered (50 ml) every 4 days and during acclimatization period before being placed in a dark room for four weeks with a relative humidity of 65% ± 10% and temperature of 18 ± 3 oC. The placement in the dark room under continuous dark condition was to simulate sea shipment of the plants. Data measured at the end of the experiment were leaf drop, chlorophyll content, plant height and plant grade on a scale of 1 = poor, not salable, 3 = good, and 5 = excellent quality. Chlorophyll content of *Codiaeum variegatum* was not measured in this experiment because of the variegated nature of the leaves.

### *2.1. Experimental Designs and Statistical Analysis*

The design of the experiment was a randomized complete block design (RCBD) with four plications in each treatment. Each replication consisted of three plants. Data was analyzed by using the analysis of variance (ANOVA). When the ANOVA showed significant ($p \leq 0.05$) F values, Duncan multiple range test (DMRT) was used to separate the means using Statistical Analysis System [9]. For regressions analysis (linear and quadratic) the formula below were adapted:

Linear: $Y = a + bx$

Quadratic: $Y = aX2 + bX + c$

### *2.2. Data Collection*

#### *2.2.1. Chlorophyll Content (mg/cm2)*

Chlorophyll content for D*racaena sanderiana* was determined using a Minolta chlorophyll meter SPAD-502, a non-destructive method. Readings were taken

from three different leaf positions (top, middle and basal) of the plants. Chlorophyll content was taken from each plant, at the end of the experiment. The following calibration equation between SPAD readings and actual chlorophyll content was used.

Y = (-0.712427) + (0.131871 x SPAD)

R2 = 0.71

*SPAD = Readings from Minolta chlorophyll meter.

Three discs, one cm2 of fresh leaves were sampled by using cork borer after the SPAD values were recorded. Samples were placed in 20 ml of 80% acetone in the dark for seven days to ensure maximum chlorophyll content extracted from the tissues. A 3.5 ml of supernatant was then sampled to measure its absorbance using a spectrophotometer at 664 and 647 nm for chlorophyll a and b content, respectively. The chlorophyll content was then calculated as follows [1]:

Chlorophyll a (mg/cm2) = {3.5/3 x (13.19 A664 – 2.57 A647) }

Chlorophyll b (mg/cm2) = {3.5/3 x (22.10 A647 – 5.26 A664) }

Total chlorophyll (mg/cm2) = chlorophyll a + chlorophyll b

*2.2.2 Plant Height*

Plants were randomly sampled for height measured from the soil surface the highest tip of the plant. The same plant was then used throughout the experiment. The average plant height was taken from measurement of three plants. This parameter was measured after every spraying of BA.

*2.3. Plant Grade*

After the final treatments, plant quality was assessed. Each plant was visually graded on a scale of 1 to 5 (1 = poor, 3 = good and 5 = excellent quality). Excellent quality plants have medium to dark – green foliage with no chlorosis or necrosis and well-formed shapes with sufficient foliage for plant. After the 4 weeks dark storage period, the plant quality was assessed.

## 3. Result andDiscussion

*3.1. Chlorophyll Content (mg/cm$^2$)*

There was a significant ($p \leq < 0.05$) interaction between BA concentration and shading duration on chlorophyll content of Dracaena sanderiana (Table 1).

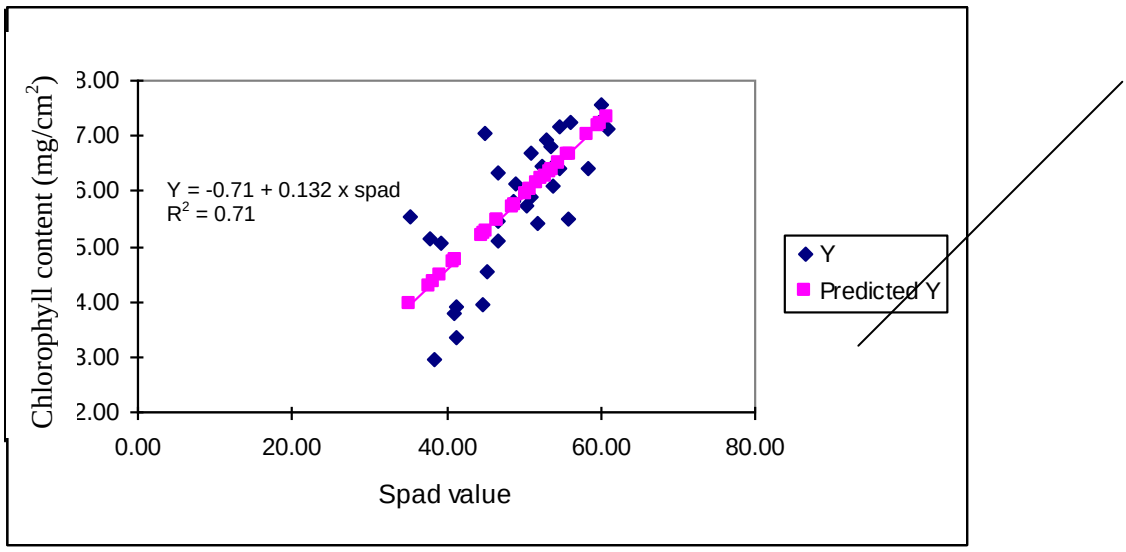


Fig. 1 Calibration graph between spad values and chlorophyll content (mg/cm$^2$).

Table 1. Effects Between Concentrations of BA and Duration of Shading on Chlorophyll Content in *Dracaena sanderiana* 4 Weeks after Simulated Shipping Conditions

| Treatment | Chlorophyll content ($mg/cm^2$) |
|---|---|
| Concentration (C) | |
| Control | 7.12 b |
| 225$mgL^{-1}$ BA | 7.40 a |
| Duration of Shading (D) (weeks) | |
| 2 | 6.74 c |
| 4 | 7.22 b |
| 6 | 7.82 a |
| Interactiom (C x D) | * |

$^{NS,*,**}$ Non significant or significant at $P \leq 0.05$, 0.01, respectively.
Note: Means with the same letter are not significantly different at $p<0.05$ (DMRT).

Chlorophyll content in the leaves of plants exposed to different shading duration depended on the BA concentration applied. In the control, the chlorophyll content increased with shading duration up to 4 weeks and then, level off (Fig. 2).

However, with plants sprayed with 225 mgL-1 BA, the chlorophyll content increased with shading duration. The effects of BA application on Dracaena sanderiana were similar with brocolli stored at 16 oC for 6 days, where chlorosis was delayed and respiration was reduced after treatment with BA [3]. [3] reported that leaf chlorophyll content of Dracaena marginata increased from 0.055 mg/cm2 in sun-grown plants to 0.081 and 0.100 mg/cm2 when the plants were grown under 40% and 80% shade, respectively, for 6 months. Shade plants have higher chlorophyll content because chloroplast and grana stacks are more highly developed under low light [3]. In contrast, [3] found that the chlorophyll content of shade plants per unit leaf area is often low.

*3.2. Plant Height Increment (cm)*

There was a significant ($p \leq 0.05$) interaction between BA concentration and shading duration on plant height increment of*Dracaena sanderiana*(Table 2). Plant

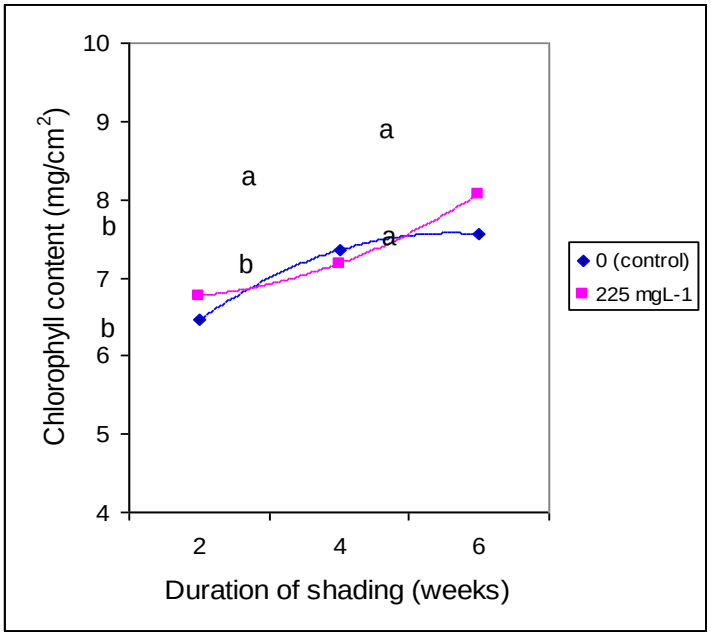

Fig. 2. Interaction effects between BA concentrations and shading duration on chlorophyll content of *Dracaena sanderiana* 4 weeks after simulated shipping conditions.

Table 2. Effects between BA Concentrations and Shading Duration on Plant Height Increment of *Dracaena sanderiana* and *Codiaeum variegatum* 4 weeks after Simulated Shipping Conditions

| Treatment | Plant Height Increment (cm) | |
|---|---|---|
| | *Dracaena Sanderiana* | *Codiaeum vairegatum* |
| Concentration (C) | | |
| Control | 2.89 a | 2.24 a |
| 150 $mgL^{-1}$ BA | - | 2.00 b |
| 225 $mgL^{-1}$ BA | 2.58 b | - |
| Duration of Shading (D) (weeks) | | |
| 2 | 2.15 b | 2.77 a |
| 4 | 3.06 a | 1.95 b |
| 6 | 2.99 a | 1.65 c |
| Interactiom (C x D) | ** | * |

BA concentration applied to *Codiaeum variegatum* and *Dracaena sanderiana* was 150 and 225 $mgL^{-1}$, respectively.

NS,*,** Non significant or significant at $P \leq 0.05$, 0.01, respectively.

Note: Means with the same letter are not significantly different at $p<0.05$ (DMRT).

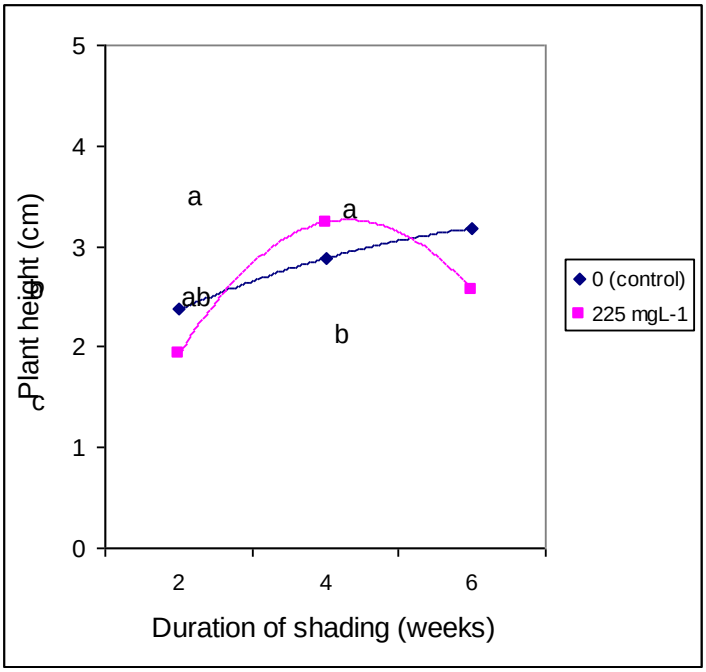


Fig. 3. Interaction effects between BA concentrations and shading duration on plant height increment of Dracaena sanderiana 4 weeks after simulated shipping conditions.

height increment of plants exposed to different period of shading depended on the BA concentration applied. In the control, the plant height increment increased with shading duration up to 4 weeks and then level off (Fig. 3). However, with plants sprayed with 225

mgL-1 BA, plant height increment increased between 2 and 4 weeks, but decreased after 4 week.

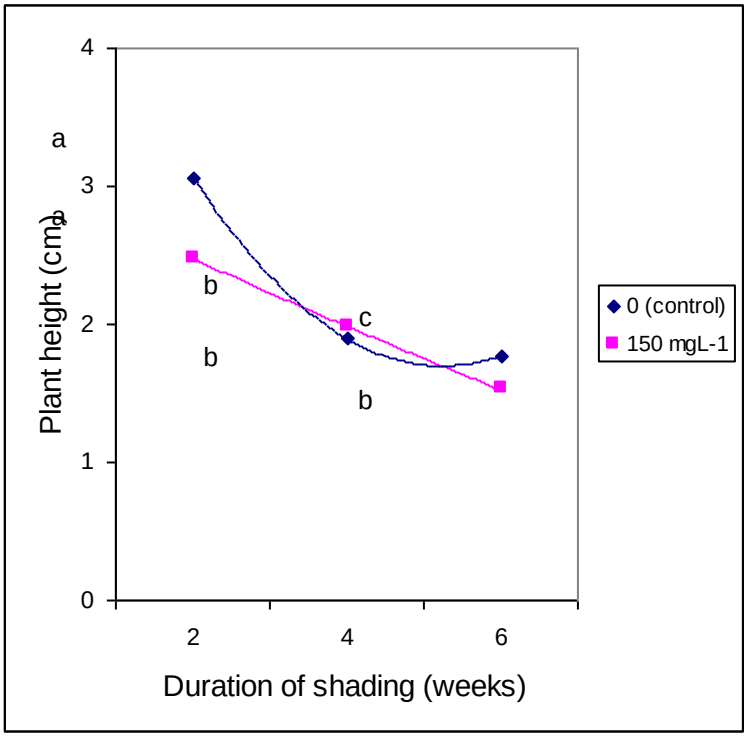


Fig. 4. Interaction effects between BA concentrations and shading duration on plant height increment of Codiaeum variegatum 4 weeks after simulated shipping conditions.

Table 3. Effects between BA Concentrations and Shading Duration on Plant Grade of *Dracaena sanderiana* and *Codiaeum variegatum* 4 Weeks after Simulated Shipping Conditions.

| Treatment | Plant Height Increment (cm) | |
|---|---|---|
| | *Dracaena sanderiana* | *Codiaeum vairegatum* |
| Concentration (C) | | |
| Control | 2.94 a | 2.50 b |
| 150 $mgL^{-1}$ BA | - | 3.78 b |
| 225 $mgL^{-1}$ BA | 3.39 a | - |
| Duration of Shading (D) (weeks) | | |
| 2 | 1.50 c | 2.00 b |
| 4 | 3.58 b | 3.67 a |
| 6 | 4.42 a | 3.75 a |
| Interactiom (C x D) | ** | Ns |

BA concentration applied to *Codiaeum variegatum* and *Dracaena sanderiana* was 150 and 225 $mgL^{-1}$, respectively.
$^{NS,*,**}$ Non significant or significant at $P \leq 0.05, 0.01$, respectively.

Note: Means with the same letter are not significantly different at p<0,05 (DMRT).

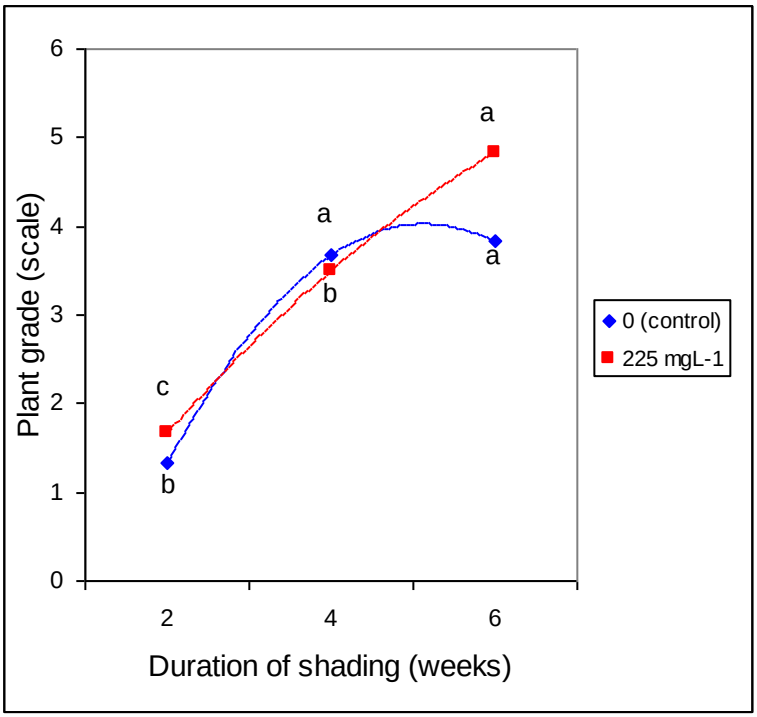


Fig. 5. Interaction effects between BA concentrations and shading duration on plant grade of *Dracaena sanderiana* 4 weeks after simulated shipping conditions.

There was a significant ($p \leq 0.05$) interaction between BA concentration and shading duration on plant height increment of Codiaeum variegatum (Table 2).

For the control, plant height increment decreased with shading duration (Fig. 4). Application of BA also decreased plant height increment up to week 4 but there was no more decrease after week 4. Shaded *Ficus benjamina* plants had longer internodes and smaller trunks [3].

*3.3. Plant Grade*

There was a significant ($p \leq 0.05$) interaction between BA concentration and shading duration on plant grade of Dracaena sanderiana (Table 3). For the control, the plant grade increased with shading duration of up to 4 weeks and then the plant grade became stable (Fig. 5). However, with plants sprayed with 225 mgL-1 BA, the plant grade increased with shading duration. Either with (225 mgL-1) or without BA plant grade increased with increase shading duration up to 6 weeks.

There was no significant ($p \leq 0.05$) interaction between BA concentrations and shading duration on plant grade of Codiaeum variegatum (Table 3). Application of BA concentration at 150 mgL-1 increased plant grade. While for *Codiaeum variegatum* shading duration of 4 and 6 weeks produced plants of the same grade but superior plants under shading duration of 2 weeks. According to [3], *Ficus benjamina* L. (weeping fig), acclimatized under shade duration for 5 or more weeks had a superior plant grade, leaf density, and leaf retention after 10 weeks indoor. Different species of foliage plants react differently to various storage conditions in terms of plant grade [3].

## Conclusion

Application of BA and shading affected the growth and plant grade of *Dracaena sanderiana* and *Codiaeum variegatum*. *Dracaena sanderiana* sprayed with BA at 225 $mgL^{-1}$ and under 6 weeks shading duration had significant reduction in leaf drop. Application of BA at 225 $mgL^{-1}$ under 6 weeks shading duration significantly ($p \leq 0.05$) increased chlorophyll content of *Dracaena sanderiana.* For *Dracaena sanderiana*, BA at 225 $mgL^{-1}$ and shading duration 4 weeks resulted in higher of plant height compared to the control. The plant grade also improved with application of BA at 225 $mgL^{-1}$.

*Codiaeum variegatum* sprayed with BA at 150 $mgL^{-1}$ before 4 weeks of shading duration reduced the percent leaf drop significantly ($p \leq 0.05$). Application of BA at 150 $mgL^{-1}$ decreased plant height increment with shading duration of 4 and 6 weeks. Application of BA at 150 $mgL^{-1}$ and shading improved the plant grade.

A large part of this industry is devoted to plants with multicolored foliage. Of 135 plant families reported to possess leaf color variegation, the genus *Dracaena* L. (Agavaceae) represents an economically important group [3]. A mayor problem in the foliage plant industry is that of transportation the plants to the consumer without quality loss [3].

## References


[1] Anderson, J.M., Goodchild, D.J. and Boardman, 1973. Composition of the photosystems and chloroplast structure in extreme shade plants. Biochim, Biophys., Acta., 325: 573-585.

[2] [3] Barbara, S.F., Lewis A.J, and Barden, J.A. 1982. Anatomy and Morphology of Sun- and Shade-grown Ficus benjamina. J. Amer. Soc. Hort. Sci., 107(5): 754-757.

[3] [3] Boardman, N.K. 1977. Comparative photosynthesis of sun and shade plants. Ann. Rev. Plant Physiol., 28: 355-377.

[4] [3] Buck, T.L. and Blessington, T.M. 1982. Postharvest effect of temperatures during simulated transit on quality factors of two Ficus species. HortScience. 17 (5): 817-819.

[5] [3] Carpenter, W.J, and Nautijal, J.P. 1969. Light intensity and air movement effects on leaf temperature and growth of shade requiring greenhouse crops. J. Amer. Soc. Hort. Sci., 94: 212-214.

[6] [3] Clark, D.G., Kelly, J.W. and Pemberton, H.B. 1991. Postharvest quality characteristic of cultivars of potted rose in respond to holding conditions and cytokinins. HortScience., 26 (9): 1195-1197.

[7] [1] Coombs, J., Hall, D.O., Long, S.P. and Scurlock, S.M.O. 1987. Chlorophyll determination. Techniques in bioproductivity and photosynthesis. Pergamon Press, Oxford. 223 pp.

[8] [3] Conover, C.A, and Poole, R.T. 1975. Influence of shade and fertilizer level on production and acclimatization of Dracaena marginata. Fla. St. Hort. Soc., 88: 606-608.

[9] [3] Conover, C.A. and Poole, R.T. 1981. Influence of light and fertilizer levels and fertilizer sources on foliage plants maintained under interior environments for one year. J. Amer. Soc. Hort. Sci., 106: 571-574.

[10] [3] Peterson, N. C. and Blessington, T. M. 1981. Postharvest effect of dark storage and light source on keeping quality of Ficus benjamina L. HortScience. 16: 81-682.

[11] [3] Poole, R.T. and Conover, C.A. 1979. Influence of shade and nutrition during production and dark storage simulating shipment on subsequent quality and chlorophyll content of foliage plants. HortScience., 14: 617-619.

[12] [3] Poole, R.T., Conover, C.A. and Ben-Jaacov, J. 1983. Long-term storage of foliage plants. Scientia Horticulturae. 24: 331-335.

[13] [3] Rushing, J.W. 1990. Cytokinins affect respiration, ethylene production, and chlorophyll retention of packaged broccoli florets. HortScience,. 25 (1): 88-90.

[14] [3] Sarracino, J.M.R., Merritt, M.R. and Chin, C.K. 1992. Morphological and physiological characteristics of Leea coccinea and Leea rubra in response to light flux. HortScience. 27: 400-403.

[15] [9] SAS Institute, 1989. SAS user's Guide, Version 6.12 Edition. SAS Institute Inc., USA.

[16] [3] Vladimirova, S.V., McConnell, D.B. and Kane, M.E. 1997. Morphological plasticity of Dracaena sanderana 'Ribbon' in response to four light intensities. HortScience. 32 (6): 1049-1052.

# ECONOMY AND SOCIAL SCIENCES

# Technical Efficiency Analysis of Poultry in the District of 50 Kota (Stochastic Frontier Production Function Approach)

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## Abstract

This study was conducted to estimate the factors that influence egg production and to analyze the production efficiency of poultry in district of 50 Kota, West Sumatra. It is based on primary data collected from 36 farmers. The model used is the stochastic frontier production function, with MLE method. The resulting estimates of technical efficiency of egg production range between 55.67% and 99.93 % with mean efficiency of 74.72%. The technical efficiency can be increased by the use of more feed ($P<0.01$). The age and knowledge of farmers does not significantly effect the technical inefficiency ($P>0.05$).

Keywords: poultry production, technical efficiency, egg production

## 1. Introduction

The farming sector is one area of rural communities' economy can be achieved through the empowering of farmers including those who raise livestock. Poultry industry has play a role in the livestock sector of Indonesia and having the potential to generate national self-sufficiency in poultry meat and eggs. It also provides a livelihood for the farmers and considerable employment opportunities within the community.

The development of poultry farming is directed toward: (a) animal protein production to maintain national food needs, (b) increasing the self sufficiency of poultry industry, (c) preserving and utilizing local resources and skills to ensure the future of poultry industry (d) creating export products that are able to compete in the international market. The goals of this development are (a) improving community intelligence and health along with encouraging market preference for safe and quality products, (b) increasing farmer income through increasing the scale of the business to an optimal level based on available resources, (c) creating viable employment opportunities throughout the region, (d) contributing increasingly to foreign exchange earnings (Badan Litbang Pertanian, 2013).

The poultry industry must be regarded as an integrated industry dependent on resources upstream and products downstream, so its development must reflect this. The success of poultry farming does not stand alone because it is influenced by upstream industries such as those related to poultry feed, provision of chicks, equipment and pharmaceuticals. Up until now, many of these upstream industries have relied on imported materials and so price fluctuations in these influence the cost of production and can fluctuated significantly. In 2013 the price of poultry feed increased from Rp 5.000/kg to Rp. 5.400/ kg if it compare to price in 2012 . This was largely because 50% of the ingredients of poultry feed are still imported. Feed companies import ingredients such as corn and fish flour from Brazil. Other countries providing raw materials are United States and Argentina. Additionally, there have been price increases in other inputs such as chicks and pharmaceuticals. Fluctuations in these prices are a barrier for farmers if they

cannot be offset by a corresponding price rise in eggs.

The district of 50 Kota has become the most important center for poultry farming in West Sumatra, with 62% of all laying hens in the province being farmed in this region. It is dominated by small to medium scale producers. The increases in production costs have not been matched by increases in egg prices resulting in a pressure on the competitiveness of poultry farming. Hence it is hard for farmers to make a profit and some go out of business. This can be seen from the population of poultry in this region reduced 27.98% from 4.858.940 to 3.499.531 between 2011 and 2012. Based on the above, this research aims to (1) analyse the factors that influence of egg production and (2) analyse the technical efficiency of egg production in district of 50 Kota.

Farell, 1957 in Kusnadi, et al., 2011 explain that efficiency can be classified into three components: technical efficiency, allocation (price) efficiency and economic efficiency. Technical efficiency (TE) indicates the relative ability of a business to obtain the maximum output from a set input at a certain level of technology. Price efficiency (PE) indicates the relative ability of a business to produce output with minimum cost at a certain level of technology. Economic efficiency (EE) is the product of TE and PE. There are two approaches to calculate efficiency, that is using an isocost curve (AA') for inputs or an isoquant curve (SS') for output. This can be illustrated graphically as in Figure 1, where , $X_1$ and $X_2$ are inputs, and Y is output.

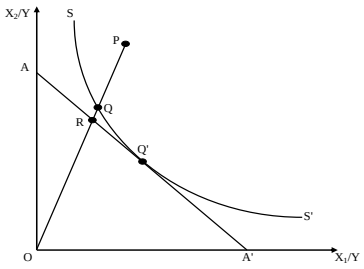


Figure 1. Technical efficiency, allocation (price) efficiency and economic efficiency (Farrell, 1957 in Kusnadi et al, 2011**)**

Kusnadi et al. (2011) explain this graph as follows, if a business's production can be placed at a point P, the distance QP indicates the technical inefficiency, that is the amount the inputs can be reduced without reducing the outputs, where point Q represents the point of maximum technical efficiency as it is on the isoquant curve. Possible reduction of these inputs to reach maximum technical efficiency is calculated as the ratio QP/OP. Price efficiency is calculated from the ratio OR/OQ. The distance RQ indicates reduction in costs that can be achieved to obtain maximum price efficiency or minimum cost. Economic efficiency is the product of technical efficiency and price efficiency. Point Q' marks maximum economic efficiency where maximum price and technical efficiency are reached simultaneously.

## 2. Research Methods

This research was conducted on poultry farming in 50 Kota district, a center of this poultry industry for West Sumatra. Primary data from surveys of thirty six poultry farming were collected by using purposive sampling. Data that were collected were: breed of hen, number of birds owned, egg production, amount of feed used and numbers of labor. A stochastic frontier model of production developed by Battese and Coelli (1992 and 1995) was used to calculate efficiency. This model can be represented matematically by the formula :

$$LnY = Ln\beta_0 + \beta_1 LnX_1 + \beta_2 LnX_2 + \beta_3 LnX_3 + \beta_4 LnX_4 + v_i - u_i$$

where:

Y : egg production (thousands eggs/month)

$X_1$ : scale of farms (number of hens)

$X_2$ : amount of feeding (kg/month)
$X_3$ : number of employees (work day equivalent of men/WDEM)
$X_4$ : dummy strain of chicken (1= ISA; 0=other strains)
$v_i$-$u_i$ : error term
$u_i$ : effect of technical inefficiency
$\beta_0$ : intercept
$\beta_1$ - $\beta_4$ : efficiency parameters for each production factor

Analysis of the stochastic frontier production function was used to determine the factors influencing egg production and the technical efficiency of the production. The following formula was used to determine technical inefficiency factors:

$$u_{it} = \alpha_0 + \alpha_1 Z_1 + \alpha_1 Z_2 + w_{it}$$

where :
$u_{it}$ : technical inefficiency
$Z_1$ : age of farmer (years)
$Z_2$ : experience of farmer (years)
$\alpha_0$ : constant
$\alpha_1$ - $\alpha_2$ : technical inefficiency parameters for each factor

Estimation of the parameters for production and technical inefficiency were calculated simultaneously using the Frontier 4.1 program (Coelli, 1986). These parameters were estimate in two stages. The first stage estimated the parameters using ordinary least squares (OLS). The second stage used maximum likelihood estimate (MLE).

## 3. Results and Discussion

### *3.1 Profile of the poultry farming in District of 50 Kota*

Profile of the poultry farming in district of 50 Kota are presented in Table 1. As can be seen from the Table 1, generally farmers are at a productive age and have adequate experiences, that is more than ten years, although the amount of experience varies considerably.

Table 1. Profile of the poultry farming in district of 50 Kota

| No | Parameter | Average | Coefficient of variation |
|---|---|---|---|
| 1 | Age of farmer (years) | 42 | 23 |
| 2 | Experience of farmer (years) | 11 | 39.81 |
| 3 | Initial scale of farms (hens) | 964 | 71.54 |
| 4 | Current scale of farms (hens) | 13,275 | 132.44 |
| | a. Number of hens in starter phase | 1,606 | 84.91 |
| | b. Number of hens in grower phase | 2,617 | 95.00 |
| | c. Number of hens in layer phase | 9,053 | 158.53 |
| 5 | Age hens begin to lay (weeks) | 18 | 3.49 |
| 6 | Age hens culled (months) | 20 - 24 | - |

Table 2. Estimation of the Stochastic Frontier Production Function using Maximum Likelihood Estimates (MLE)

| Variable | Symbol | Parameter Estimation | t-ratio |
|---|---|---|---|
| Constant | | -2.537 | -23.347 |
| scale of farms (number of hens) | $X_1$ | -0.035 | -1.137 |
| Feed consumption (kg/month) | $X_2$ | 1.158 | 22.684 |
| Number of employees (WDEM) | $X_3$ | -0.045 | -0.730 |
| Strain of hens | $X_4$ | -0.023 | -0.660 |
| Log-likelihood OLS | | 31.196 | |
| Log-likelihood MLE | | 32.899 | |
| LR | | 3.405 | |

This bodes well for the future of the industry in providing family livelihoods. This is further indicated by the majority of farmers beginning their business at a young age, that is 20 years. Farmers began their farms with an average of 964 laying hens, but at the time of the research this had grown to an average of 13,275 hens, and farm size ranged from 2,000 to 80,000 hens. Generally ISA and Loghman strain were raised. They stared laying at an average of 18 weeks and continued to produce until they were 20 – 24 months old.

*3.2 Estimation of the Egg Production Function*

The variables that influence egg production are estimated by using the stochastic frontier production function. During the estimation process all the factors that were considered as possible influences on production were included in the model as listed in the stochastic frontier function in the methods section. The resulting parameters for these variables are set out in Table 2. The values of sigma squared ($\sigma^2$) and gamma ($\gamma$) are 0.009 and 0.999 respectively. Gamma is an indication of technical efficiency in the production process.

The value of the ratio generalized likelihood (LR) from the Stochastic Frontier production function is 3.405 which is smaller from 10.371, the critical value as listed in Kodde dan Palm (1986), the value of $\alpha = 5\%$ indicates that there is no technical ineficiency effect in the model using this technology. Only one variable in the production function appears to have significant influence ($P<0.01$) on production, that is feed consumption. The scale of the farms, the number of employees and the strain of hens used do not have a significant effect ($P>0.05$) on production. Hence, feed consumption appears to be the determining factor in increasing technical efficiency. The research shows that, on average feed consumption is 32.89; 93.61; and 122.91 g/hen/day for starter, growers and layers phase respectively. By the level of feed consumption, average hen day production is 79.11%. Andri, et al. (2011) obtained similar results that feed consumption had a significant effect in determining egg production.

3.3 Technical Efficiency of Egg Production

Technical efficiency was analysed from the stochastic frontier production function. The value of the technical efficiency is categorized as efficient if it is the same or bigger than 0.7. Analysis shows that the average of technical efficiency is 0.7472.

This indicated a productivity of 74.72% which means there is a possibility to increasing production by 25.28 to reach maximum efficiency if optimal practices are utilised. Egg production is however efficient as productivity is above 70%. This result indicates that poultry farming in this area is highly developed considerably more efficient than in the Delta-Nigeria where Alabi and Aruna (2005), measured technical efficiencies from 0,09 to 0,63, with an average of 0,22. The spread of technical efficiency values from the 36 poultry farming is presented in Table 3.

Table 3. Distribution of technical efficiency values of poultry farming in district of 50 Kota

| No | Technical Efficiency | Number of farms | Percentage |
|---|---|---|---|
| 1. | < 0.70 | 7 | 19.45 |
| 2. | 0.70 - 0.79 | 23 | 63.89 |
| 3. | 0.80 - 0.89 | 4 | 11.11 |
| 4. | ≥ 0.90 | 2 | 5.55 |
| | Total | 36 | 100.00 |

Table 4. Estimate of technical inefficiency in poultry farming in district of 50 Kota

| Variable | Estimated Value | t – ratio |
|---|---|---|
| Constant | 0.125 | 0.986 |
| Experience of the farmer ($Z_1$) | 0.003 | 0.518 |

| | | |
|---|---|---|
| Age of the farmer ($Z_2$) | 0.003 | 1.154 |

It can be seen that 80.55% of the poultry farming have a technical efficiency more than 0.70, that is they can be categorized as having a good technical efficiency. The level of technical efficiency can be interpreted in two ways. On the positive side; high technical efficiency can reflect sound management with well informed decision-making based on an adequate understanding of the influence of the factors involved in egg production.

Negatively, current high technical efficiency indicates that there is little opportunity to improve production as the discrepancy between efficiency achieved and the maximum possible based on best practice is already small. For real improvement in production to occur, new technological inovations are needed. If current technical efficiency was smaller then the opportunity to improve production toward the maximum achievable would be larger. Table 4 shows the factors that influence technical inefficiency in poultry farming in district of 50 Kota.

The experience of the farmer ($Z_1$) appears to have a small positive but insignificant impact on technical inefficiency ($P>0.05$). The age of the farmer ($Z_2$) also has a small positive but insignificant impact on technical inefficiency of the business ($P>0.05$).

## Conclusion

The following conclusions can be drawn from the results of the study:

1. Poultry farming in district of 50 Kota has good technically efficiency with an average value of technical efficiency is 74.72% and hen day production is 79.11%.
2. Feed consumption is the one factor that significantly influences the technical efficiency, hence the farmer must pay primary attention to the quantity and quality of the feed rations given to the hens.
3. The level of technical inefficiency in egg production is not significantly effect by the age and the experience of the farmer.

## Acknowledgements

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## References

Alabi, R.A and M.B. Aruna, 2005**.** Technical Efficiency Of Family Poultry Production in Niger-Delta, Nigeria. *Journal Central European Agriculture, Vol. 6 (2005) No. 4 p. 531-538.*

Andri, R. Wati., A. Suresti 2011. Faktor-Faktor yang Mempengaruhi Pendapatan Peternak Ayam Ras PetelurDi Kecamatan LarehSago Halaban Kabupaten Lima 50 Kota. *Jurnal Peternakan Indonesia. Vol 13 (3) : 206-214.*

Badan Litbang Pertanian, 2013. Statistik Badan Litbang Pertanian 2013. Kementerian Pertanian RI.

Battese, G. E. and T. J. Coelli. (1992). "Frontier Production Functions, Technical Efficiency and Panel Data: With Application to Paddy Farmers in India."*Journal of Productivity Analysis 3, 153–169.*

Battese, G. E. and T. J. Coelli. (1995). "A Model for Technical Inefficiency Effects in a Stochastic Frontier Production Function for Panel Data."*Empirical Economics 20, 325–332.*

Coelli, T.J., 1986. Aguide to Frontier Version 4.1: A Computer Program for Stochastic Frontier Production and Cost Function Estimation. Centre for Efficiency and Productivity Analysis. UNE, Armidale.

Kodde, D.A. and F.C Palm, 1986. Wald Criteria for Jointly Testing Equality and Inequality Restriction. *Econometrica, Vol. 54, Issue 5, p. 1243-1248.*

Kusnadi, N, N. Tinaprilla, S.H. Susilowati, dan A. Purwoto, 2011. Analisis Efisiensi Usahatani Padi di Beberapa Sentra Produksi Padi di Indonesia. *Jurnal Agro Ekonomi Vol. 29 (1), Mei 2011.*

# Teaching Accounting In Business School: A Personal Reflection

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**Abstract**

Accounting is one of compulsory subjects in business school. This article describes reflection of the author after almost ten years teaching accounting in business school to undergraduate and graduate level. The focus is on teaching first year students about how to encourage them learning accounting as the first step in understanding business as a whole and finance in specific. Motivating students is as important as delivering the subject in a more simple way. Lecturer's exposure on business, as well as understanding on millennial generations and usage of technology-based learning will assist learning process to enable students to gain more insights. However, as learning is a process, lecturers should never stop finding a more appropriate way of teaching accounting.

*Keywords*: accounting, learning, business school, millennials, technology

*If you can't explain it simply, you don't understand it well enough.*
Albert Einstein[1]

## Introduction

Accounting, as one of compulsory subjects in business school, is usually taught at the first year in business school to both undergraduate and graduate students, especially management students. In this case, teaching accounting is challenging because lecturers have to deal with students whose no or less exposure of business since they have not had sufficient work experience nor work experience. In this paper, I would like to share my personal journey in teaching introductory accounting to business students in both undergraduate management programme and master of business administration (MBA) programme.

### Introducing Accounting to Business Students

Several years ago, a high school teacher asked me how to make accounting more understandable by the students. In my opinion, the answer is by relating as close as possible accounting subject taught in the class by the business/management context. Learning accounting in high school may somewhat technical that students may not capture the context where accounting operates. In fact, understanding business/management is important that students get the broader picture to understand accounting as a language of business and how accounting contributes to decision making. When accounting is only one subject of many taught subjects, it might not have enough time to explore more on the nature of business of a company or an industry. Therefore, higher education should fill this gap when offering accounting subject to the students.

Starting first day with high school graduates in their first accounting course might be of particular experience. It might be some of them do not know accounting is. We might meet persons that have little idea about what they should do in university. Some of them join business school because of certain reasons. There are those who enter business

school as their own choice to be business professionals or entrepreneurs. On the other hand, some students join because they have to since it is obliged by their parent or because it is the only an available choice at the moment as they are not accepted in other universities. Following their friends instead of their own consideration is also one of the reasons. On the other hand, faculty members should deal with the image of accounting as difficult and dull subject as perceived by students. Hence, it is not a surprise when we find students not paying full attention to class whereas students are expected to understand accounting as a language of business. Consequently, it is a challenge to bring up the accounting course in the class.

Understanding Millennials

Classroom learning allows social interactions and mutual understanding among class members including the faculty member [2]. What we might usually miss is to undertand who our students are. We often compare ourself, a person with personal learning experience in university, with them. As a matter of fact, our students are coming from Millennials generations who were raised in a protective way by their parents [3]. They feel confident and want to achieve high [4]. In addition, they adore themselves as being special and tend listen more to their friends than to other people, including their parents. They often listen to what they think it is worth and part of them. For example, I found that they respect teachers in class because he or she is considered being one of them not because of solely being a teacher. It is somewhat different to what happened many years ago when we felt reluctant to teachers and respected whoever standing in front of the class. As the millennials communicate via social media and enjoy communities they tend to be more egalitarian and affect the way they communicate to other people. If they always ask why and speak in a more frankly way, it does not necessarily mean they are impolite.

Delivering Accounting Course to Millennials

Once the millennials' traits are understood, the next step is to deal with those traits despite winning through them. Accounting is often regarded as a hard and monotonous subject by students. This label creates presumption that block learning process. Lecturers can overcome this by delivering a good impression at the beginning of the class sessions. At the first meeting, it is considerable to focus on the class rule, do and don't, approved by all the class members; for example the use of handphone during the lecture and how to deal with latecomers. Actually, Millennials accept authority and follow the rules which are considered reasonable [3]. Lecturer should tell his expectation and asked students' expectation toward the subject. It is important that the whole class have clear expectations and detailed instruction. They have to understand what the course is, how, and where to go. It is necessary to build a rapport between lecturers and the class members.

When the students show their understanding, it is the time to introduce the course. The first thing is to raise their interest in acconting. I usually begin with the big picture of management functions in organization and how significant is its contribution to business organization. In this part, knowlege and previous work experience of a lecturer is helpful to enable students to gain broader insights. The various accounting profession and its career path are also introduced particularly to accounting students. I can say that at the first meeting, most of time is used to build a rapport and present the course objective. The essence is to encourage students to learn while the content will start from the following session until the end of class.

The smooth first session will lessen the students' barrier in learning especially because of their initial perception of accounting. The next challenge is how to maintain the motivation of students. I suggest

that accounting rules and techniques delivered in a more simple way. To gain a more simple approach, not only do lecturers derive benefit from teaching materials but also from their own knowledge and experience.

For instance, teaching accounting often starts with introduction of double entry system of debit and credit. As an alternative, lecturers may begin with and emphasize the use of accounting equation instead of double entry book keeping techniques. Most students tend to memorize debit-credit position in journal entry, so it is better to use accounting equation to give the logic of accounting system. Even the mature students like MBA students face difficulties in understanding debit and credit system. Accounting equation should be reviewed in each topic to remind the students of its importance until the end of course. In addition, the use of daily example is effective in explaining conceptual framework of accounting. A situation when a theater receives advance cash payment and audience buy the ticket before the move plays is a good illustration in explaining such accounting principles as going concern, periodicity, revenue recognition and matching revenue against cost.

The more detail the accounting techniques, the more the students get lost in lecture. Lecturers can attract their attention and bring back their focus by using their knowledge from current and previous work experience. Take a topic of non-current assets for example, industrial revolution in the 18th century in England can be used as a background. The acquisition of Whatsapp by Facebook is a good example which raises students' interest as most of them are social media users. Work experience in industry also helps explain the nature of accounting in MBA class. For example, when delivering the topic of financial statement analysis, a lecturer can illustrate about how managers used financial report for performance analysis. A description of how accounting capture and support business process is important to highlight. However, not all of us are good story tellers, thus it is advised to use other learning sources from internet such as Youtube and play it in class.

By understanding accounting and its context, students can relate the relevance of accounting to other financial and non-financial subjects, particularly management functions subjects in the following semesters, such as financial management, marketing management, operation management, human resources management. Research has found a significant relationship between accounting performance and performance in non-core accounting business related subject [5]. Additionally, Lecturers can take advantage of their own research to enrich the content of teaching. In internal control topic for instance, a lecturer whose research interest in corporate governance can put in his research findings to strengthen his topic delivery. At this point, lecturers may experience less difficulty in teaching MBA class because the students might have accounting exposures from their undergraduate study or work experience.

Giving class assignment is one of the media to give students learning experience. Faculty can spare half of class duration for class assignments and allow for a discussion. The materials can be taken from textbook, self creation, or other sources. This will assist students from all types of learning. Students with abstract conceptualization will benefit from class discussion to strengthen the concepts; whereas students with concrete experience will capture the concepts by learning experience on the spot [6]. In addition, a lecturer may ask for a project which require fieldwork to enable students to look at the application of accounting concepts in business practice. For instance, in my managerial accounting class, I require students to conduct an observation to a company in their production cost accounting system. What I expect in the end of semester is that students can see the best practice and draw a better image of accounting. The

students are encouraged to learn more on advanced accounting course in the following semesters, able to its connection to other subjects in business school and sense its application in industry.

Taking Advantage of Technology

Millennials live in a more convenient environment and depends on technology in making contact with people [3]. In most cases, they are so addicted that they cannot take themselves off their gadget for social media. It is a common now in class where some students are busy with their gadget rather than paying attention to lectures. While it needs a consensus of using gadget in class; however, lecturers can derive benefit from technology for example online learning to enhance learning experience. If faculty cannot create his own learning system, they can benefit from web-based learning materials provided publishers which usually supplement the textbook. For example, John Wiley & Sons provides WileyPLUS which complements its accounting textbooks while McGraw-Hill offers Connect. Such web-based learning will complement class assignment allow learning flexibility to students [6]. Students can gain from not only reading materials but also videos. Online resources facilitates different learning styles of students. Students with abstract conceptualization will find more readings to fulfill their curiosity while students with concrete experience might be attracted to learn more by exploring online [6]. Lecturers also benefit from hundreds or thousands exercises available as homework to students. Deadline of assignment can be arranged. The automatic system of grading for multiple choice-type questions saves time and energy. Consistent exercise by homework via web-based learning is considered the most important element to succeed in the accounting course [7]. The web-based learning is found to be effective in increasing learning efficiency since the students have had immediate feedback after finishing every exercise. This will allow a student-centered learning with supervision of faculty member [7]. System quality and information quality of web-based learning are predictors for the success of online learning implementation [8]; though, the role of instructors should not be neglected. Although online learning does not necessarily require face-to-face interaction, its advantages cannot be gained without a close relationship established in the class between students and faculty members and also among students [2]. To enable this, a lecturer should act as a learning facilitator and are available to be in touch with students. However, research shows mixed result about web based learning's significant effect on learning outcomes [7] so that subsequent research should be conducted to investigate the effectiveness of the web-based learning, especially those provided by major accounting textbook publishers. Implementing the web-based resources as a textbook supplement is not without a problem. As lecturers put online exercise for homeworks as a component of total scores together with exams, class participation, quiz, and term paper; some students complain that they feel overwhelmed because they have to purchase and they cannot borrow from their senior or from campus library. It is because each book only provides a single access that can be used once after activation for a certain period of time, usually for one semester. Nevertheless, lecturers should find innovative ways to create a supportive learning environment which suits their own teaching style.

## Conclusion

The paper objective is to reflect a personal journey in almost ten years teaching accounting. Conducting an introductory accounting course is not only about teaching accounting techniques but also about motivating students to learn. From the first session until the end, lecturers should keep encouraging students to explore more on the subject so that the students can learn

accounting in its context. Motivating students is as important as delivering topics in a more simple approach. Gaining a more simple way of delivery begins with knowing students' profile, adapting with them, and taking advantage of both working experience in industry and academic research. Web-based materials from publishers help faculty member create a more conducive learning experience that suits Millennial generations. The point is to relate accounting lesson be as close as possible to business/management as its contexts. Thus, in the long term, all of these strategies create a positive attitude toward accounting among students to keep students follow the lesson from the beginning until the end of study. At last, as learning itself is a continuous process, lecturers should never stop searching more innovative and convenient methods of teaching accounting.

## References

[1] (2016) BrainyQuote website. [Online]. Available:https://www.brainyquote.com/quotes/authors/a/albert_einstein_3.html

[2] A. Y. Ni, "Comparing the effectiveness of classroom and online learning: teaching research methods", *Journal of Public Affairs*, vol. 19, no. 2, pp. 199-215, 2013.

[3] N. Howe and W. Strauss, *Millennials* Rising *: The Next Great Generation*, New York, USA: Vintage Books, 2000.

[4] S. Williams, J. Beard, and M. Tanner, Coping *with Mellennials on Campus*, BizEd July/August 2011.

[5] R. L. Baker, W. E. Bealing, Jr., and E. Pitingolo, "Does performance in a student's first accounting course provide insight into performance in other business core courses?' in *Northeast Decision Sciences Institute*, 2010.

[6] H. Lu, L. Jia, S. Gong, and B. Clark, "The *relationship* of Kolb Learning styles, online learning behaviors and learning outcomes", *Journal of Educational Technology & Society*, vol. 10 no. 4, pp. 187-196, Oct. 2007.

[7] G. H. King and S. Mo, "The application of web-based learning in a managerial accounting course", *Academy of Educational Leadership Journal*, vol. 17, no. 4, pp. 53-62, 2013.

[8] S. B. Eom, "Effects of LMS, self-efficacy, and self-regulated learning on LMS effectiveness in business education', *Journal of International Education in Business*, vol. 5 issue 2, pp. 129-144, 2012.

# Analysis Influence of Technical Competence on Company's Performance In Electrical Engineering Company In Bandung

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## Abstract

One of the industry sectors that have an important role in the era of globalization is the electro engineering sector. The era of globalization led to intense competition. One of the negative effects of the intense competition is declining corporate profits. Drop in profits caused many companies to take action to reduce their employees without seeking a factor of declining profits in detail. Whereas, employee is the important resources to maintain competitive advantage. Competitive advantage can be measured by the performance of which is owned by the company. The company's performance can be formed of competence that is unique, rare, irreplaceable, and difficult to imitate owned enterprises, one of them is the competence of the individual. According to a competency-based approach and the resource-based approach, individual competence is affect the performance of the company is technical competence. Questionnaire is built based on the dimensions of the company's performance and technical competence,are processed using partial least squares application. The results of this study indicate that the technical competence positively affect corporate performance with moderate bond.

Keywords: resource-based view, competence-based view, competence, technical competence, company performance, partial least square

## 1. Introduction

One of the industry sectors that have an important role in the era of globalization is the electro technical sector. The era of globalization led to intense competition. One of the negative effects of the intense competition is declining corporate profits. Drop in profits caused many companies to take action to reduce their employees without seeking the cause of the decline in profit factors in detail. Whereas, the employee is one of the necessary resources to maintain a competitive advantage. According to a competency-based approach and the resource-based approach, one of individual competencies that affect the performance of the company is technical competence [6]. Individuals who have a vital role is at the level of middle management employees such as managers, senior managers, and the general manager [6][10]. The company used as a research site is state-owned enterprises in the field of electrical engineering and its subsidiaries in Bandung. Models built in this study consists of the dimensions of corporate performance and technical competence to act as second order latent variables. Dimensional performance of the company consists of three indicators, namely market share, sales growth, and customer satisfaction that were acted as first order latent variables [2][8]. While the dimension of technical competence is composed of two indicators technical skills [3] and contextual knowledge [1] that acts as a second order latent variables. The purpose of this study was to confirm the effect of the technical competence of the company's performance in state-owned enterprises in Indonesia.

## 2. Material and Methods

### *2.1.Resource-Based View*

Resource-based view emphasizes the importance of internal resources to maintain a competitive advantage. This view proved that the company's performance is a function of how well the manager to build the company by using the resources of high value, rare, not easily imitated, and difficult to replace. The ability of the manager is a resource that is not easily replicable and related to company performance [9].

### *2.2.Competence-Based View*

Competence-based view of placing human beings as the center of attention and emphasizes the importance of human resources to achieve corporate objectives. Therefore, the competence became general discussion on human resources systems to compare humans with other resources you need. This view helps companies to identify the knowledge, skills, attitudes, and capabilities that are required in certain occupations to align corporate strategies and priorities [12].

### *2.3.Competence*

Competence can be seen as an input or output on human behavior [12]. Competence is defined as the ability to maintain the coordination of the deployment of assets as the basis for achieving the company's goals. Taking into account the cognitive aspect and the aspect of action, competence is defined as the ability and the specific skills of the company in the development of their resources by considering the cognitive characteristics to achieve the desired objectives of the company [3]. Competency concept consists of the company's competency and personal competence. Competence of the company is a combination of skills and knowledge that sticks in the systems and processes in companies that were absorbed by all the members and structure of the company and always there. Whereas, personal competence is a combination of experience, knowledge, and skills possessed by an individual or group of individuals [4].

### *2.4.Technical Competence*

Technical competence is defined as understanding in the field of Technology, technical equipment and engineering work, product applications, the evolution and direction of technology, and relationships with assistive technologies [6]. Technical competence is important for managers to be able to work effectively. Literature which describes the dimensions that measure the technical competence is not found then the dimensions of technical competence derived from research [6] and a study that used a view based on competence. From the translation of that study concluded that technical competence is formed of two dimensions, technical skills and knowledge that is unique, irreplaceable, rare, and limited which is owned by a manager. Two dimensions is measured using indicators technical skills of research [3] and knowledge of research [1].

### *2.5.Company's Performance*

A multidimensional approach is used to determine the dimensions that make up the company's performance [8]. The dimensions consist of market-based performance, financial performance, and customer satisfaction. Market-based performance is measured using indicators of sales growth and market share growth. Financial performance indicators are measured using the total cost reduction, return on investment, return on assets, financial liquidity, and net profit. Customer satisfaction is measured using indicators of the reduction of response time for product design changes, the reduction of response time for product for product volume changes, the accuracy of order processing for customers, the reduction of product return ratio, the speed of order handling, and the reduction of response time for product return. However, the financial dimension is not used

in this study taking into account the difficulty of getting financial data from the company.

*2.5.Partial Least Square*

Structured equation modeling (SEM) is one of the multivariate statistical methods to explain the relationship between latent variables based on the variable observed [11]. SEM method has a high degree of flexibility for researchers to connect between theory and data. SEM consists of two types of covariance-based SEM and variance-based SEM (Partial Least Square). Comparison of the two types of SEM methods are described in Table 1.

PLS is an analysis method that can be relied upon because it does not assume the data must use a scale of particular measurements and small sample size. Determination of the number of samples in the method PLS is (1) ten times the number of the biggest indicators of reflexive or (2) ten times the amount of the biggest indicators of reflexive or (3) to ten times the largest number of lanes structural directed to construct certain structural model [4]. The PLS model evaluation can be done in two ways, namely the evaluation of the measurement model and structural model evaluation.

Table 1. Comparison of the two types of SEM methods

| Criteria | CB SEM | Partial Leas Square |
|---|---|---|
| Objective | Parameter Oriented | Prediction Oriented |
| Approach | Covariance | Variance |
| Assumption | Parametric | Non-parametric |
| Parameter estimates | Consistent | Consistent |
| Implication | Optimal for parameter precision | Optimal for prediction precision |
| Model complexity | small-medium | Large |
| Sample size | 200-800 | 100-1000 |
| Latent variable score | Indeterminate | Explicitly estimated |

Fig. 1. Structural Model

*2.6.Research Methodology*

*2.6.1.Preparation Research Instrument*

The research instrument used in this study was a questionnaire enclosed. Questionnaire established based on the operationalization of latent variables, dimensions / variables manifest and latent variables statement items of technical competence and performance of the company. Scale used in the questionnaire is Likert scale consisting of six ratings points. Scoring points for the variable performance of the company is very low, low, low enough, high enough, high, and very high. Scoring points for technical competence variables are strongly disagree, disagree, somewhat agree, quite agree, agree, and strongly agree.

*2.6.2..Identification of the Population and the Respondent*

The population of this study is the middle level managers and senior managers of the company from state-owned enterprises in the field of electrical engineering and infrastructure. An overview of the study population are shown in table 2.

Table 2. Population of Respondent

| **Company** | **Manager** | **Senior Manager** |
|---|---|---|
| PT. LEN | 17 | 3 |
| PT. SEI | 3 | - |
| PT. ELTRAN | 4 | - |
| Total | 24 | 7 |

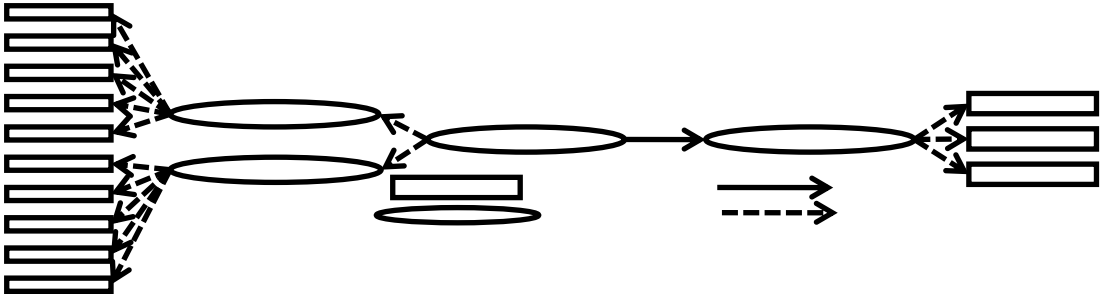

Fig. 2. Measurement Model

*2.6.3. Determining the Sampling Method*

The sampling method used is non-probability sampling, by using purposive judgment sampling. This method is used because samplers provide equal opportunities for every member of the target population to be a respondent. Purposive technique used for samples taken pre-defined criteria-criteria with the best position to provide the required information. Samples in this study were managers and senior managers at companies in the electrical engineering industry which is a state-owned enterprise.

The minimum sample used in this study followed the equations raised by Ghozali [5], which is ten times the amount of structural path. The number of structural path in this study is one that is the path that leads to the variable performance of the company. Therefore, the minimum number of samples used is ten respondents.

*2.6.4.Data Collection*

Data collected in this study consisted of primary data and secondary data. The primary data obtained from questionnaires while the secondary data obtained from historical data of the company.

*2.6.5.Data Proccessing*

Raw data from the results of questionnaires was processed using SPSS 13 and SmartPLS 2.0 to determine the validity and reliability of the questionnaire and the statement of the questionnaire. Questionnaire statement declared valid and reliable then be processed using SmartPLS 2.0 to perform path analysis, analysis of measurement model and structural model.

*2.6.6.Analysis*

Analysis used in this study is a qualitative and quantitative analysis. Quantitative analysis based on the analysis of validity, reliability, and path analysis. Qualitative analysis is based on a comparison between the test results with theoretical models of research on past research.

## 3. Result andDiscussion

First, the validity of the test conducted on the entire item statement to view the accuracy in measuring the dimensions statement items that make up the technical competence. Then, reliability testing is done to see consistency in measuring the dimensions of the items in different conditions. Statement items considered valid if it has a loading factor and Spearman's rho coefficient greater than 0.5 and the dimensions of each latent variable has a value said to be reliable if the Cronbach alpha and composite reliability greater than 0.6.

Based on reliability test, the dimensions of contextual knowledge and technical skills that affect corporate performance are reliable. Based on test validity, the item statements that make up dimensions contextual knowledge is validentirely and there is one item on the statement of technical skill dimensions that are not valid. Cronbach alpha values, composite reliability, speraman's rho and factor loading of each dimension and statement items described in Table 3.

Table 3. Cronbach Alpha (CA), Composite Reliability (CR), and Factor Loading (FL)

| Dimensions | CA | CR | Item | FL |
|---|---|---|---|---|
| Contextual Knowledge | 0,862 | 0,902 | CK1 | 0,7877 |
| | | | CK2 | 0,7114 |
| | | | CK3 | 0,7155 |
| | | | CK4 | 0,8556 |
| | | | CK5 | 0,6697 |
| Technical Skill | 0,790 | 0,861 | TS1 | 0,7063 |
| | | | TS2 | 0,7590 |
| | | | TS3 | 0,7397 |
| | | | TS4 | 0,3202 |
| | | | TS5 | 0,5256 |

After the validity and reliability tests conducted on the items that make up dimensions statement of technical competence then forwarded by testing the structural model. Testing the structural model is done by observing the path coefficient of all latent variables and dimensions.Structural models were evaluated using R square and t test for dependent latent variables. Structural model evaluation is done in two stages. First, the evaluation is done by considering the relationship between the dimensions of first order with latent variables second order, namely the relationship dimension of technical skill and contextual knowledge of the technical competence. Second, the evaluation is done by considering the relationship between the latent variables technical competence and performance of the company's.The relationship between latent variables and relationships between the latent variables with dimensions presented in Table 4.

Path coefficient value (PC) having a value greater than 0.5 and t-statistics values greater than 2.074 indicate a significant and positive effect given by the dimensions to the latent variables or latent variables independent to the dependent latent variables. R-square value indicates the observed values of models and parameter estimates in the model, the greater the R-square value, the better the value of observation and estimation of the parameters of the proposed model.

Table 4. The relationship between latent variables and dimensions

| Latent variable | $R^2$ | Dimension | PC | t-stat |
|---|---|---|---|---|
| TC | 0,305 | CK | 0,933 | 83,73 |
| | | TS | 0,795 | 27,21 |
| CP | 0,344 | TC | 0,490 | 6,021 |

## Conclusion

Conclusions of this study is the technical competence have a significant influence and positive impact on the company's performance in the company's state-owned enterprises. It indicates a model built to confirm the relationship between the technical competence of the company's performance in the company's state-owned enterprises have no difference with the model of the referenced based on previous research. On previous research that made reference population and samples taken are private companies. Respondents considered the technical competencies are competencies that must be owned by the managers at the middle management level and above, in order to improve company performance.

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## References

[1] Brill, J.M., Bishop, M.J., and Walker, A.E. (2006). The Competencies and Characteristics Required of An Effective Project Manager: A Web-Based Delphi Study, *Association for Educational Communication and Technology.*

[2] Coombs, J.E., Bierly III, P.E. (2006). Measuring Technological Capability and Performance, *R&D Management Journal*

[3] El-Sabaa, S. (2001). The Skills and Career Path of An Effective Project Manager, *International Journal of Porject Management.*

[4] Esrig-Tena, A.B., Bou-Llusar, J.C. (2005). A Model for Evaluating Organizational Competencies: An Application in The Context of

Quality Management Initiative, *Journal of Decision Sciences.*

[5] Ghozali, I. 2008. *Structural Equation Modellin: Metode Alternatif dengan Partial Least Square.* Semarang, Badan Penerbit UNDIP.

[6] Grant, K.P., Baumgardner, C.R., Shane, G.S. (1997). The Percieved Important of Technical Competence to Project Manager in The Defence Acquisition Community, *IEEE Transaction on Engineering Management.*

[7] Hakim, R.B.R. (2010). *Development of Customer Satisfaction Model of Syaria Bank by Considering Service Quality and Syaria Principle*. Thesis of Industrial Management Engineering Program, ITB, Bandung.

[8] Kim, S.W. (2009): Investigation on Direct and Indirect Effect of Supply Chain Integration on Firm Performance, *International Journal of Production Economics*.

[9] King, A.W., and Zeithaml, C.P. (2001). Competencies and Firm Performance: Examining the Causal Ambiguity Paradox, *Strategic Management Journal*.

[10] Lado, A.A., Boyd, N.G., and Wright, P. (1992). A Competency-Based Model of Sustainable Competitive Advantage: Toward A Conceptual Integration, *Journal of Management*.

[11] Mukti, I.Y. (2010). *ERP management in the post-phase project and its impact on the realization of the benefits of ERP*. Thesis of Industrial Management Engineering Program, ITB, Bandung.

[12] Wickramasinghe, V., and De Zoyza, N. (2009). An Assessment of Managerial Competency Need: Empirical Evidence from Sri Lankan Telecommunication Service Provider, *International Journal of Human Recources Management*.

# Reflections Of Social Reality In The Activities Of Women Trafficking In West Sumatra

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## Abstract

Women trafficking is a kind of activity in which women are not regarded as human. The women are treated as stuff in which they can be sold and bought. This reality occurs around us since women trafficking is like the peak of ice-mountain. The peak looks tiny but the foot is very large. Many people do not know well this phenomenon. They even do not realize that they might be part of this cruel activity. This article then tries to explain the social reality reflected in the phenomena of women trafficking activity. The research is conducted in West Sumatera (Tanah Datar and Lima Puluh Kota District) The data are collected by observational method, interview and note-taking technique. The analysis is done by referential and pragmatic identity method related to the concept of women trafficking by Wheaton et al (2010) and Oktavianus and Revita (2013). The result of analysis is descriptively presented. Having analyzed the data, it is found that there are four social realities reflected by the activities of women trafficking. They are (1) dishonesty; (2) money-oriented; (3) consumptive; and (4) carelessness.

Key words: social reality; women trafficking; activity, West Sumatra

## 1. Introduction

Women trafficking is a kind of the cruelest action in this world. Billions of women become the victim of this trafficking. They are treated like slaves, exploited, raped, and even killed. References [1] estimated 27 million people are trafficked every year and 55% are women. The fund supporting this activity is around 32 billion USD. This illicit business is really tantalized [2]. (Wheaton, SchauerdanGaldi, 2009).

The business of women trafficking is the third biggest illegal business in the world after weapons and drugs [3]. The low risk but high profitbusiness gets increased and increased from time to time. Some countries have become the main source and target of women trafficking. One of them is Indonesia.

The problem of women trafficking has reached the alarming situation. This is indicated by several aspects such as (1) the high mobility of women; (2) the increase of the number of man power supply company; (3) the high percentage of the fraud case [4].

This women trafficking is like the ice mountain which is camouflaged quantitavely. Some actions done by the government does not stop the activities of this women trafficking. It even gets wider and wider. Various modes become the strategy for trapping the women becoming the victims, like the job vacancy with high salary, lending money, kidnapping, and fake marriage.

The above phenomena reflects certain social reality. This writing then is trying to describe the social realities reflected in the activities of women trafficking. The aim is at

identifying the realities in social life related to women trafficking.

## 2. Material and Methods

### *2.1. About Women Trafficking and Social Realities*

Based on the law of No. 21 year 2007 and UN Protocol about human trafficking, it is described that trafficking is a kind of activity starting from recruitment, transporting, reception, sending, removing, or accepting someone under threating, violating, kidnapping, incarcerating, doing folgery, or persuasing to get a lot salary, or in convenient place. The activity of women trafficking is not impossible conducted by those who are familiar with the victims to be. They might be their own family, their villager-mate, or even their parents.

This happens due to some factors. Some of them is economic, social, and psychological factor [5]. These factors cannot be overcome. In the sense that, acitivities of women trafficking is getting and getting bigger from time to time.

The women trafficking is like the ghost that cannot be easily identified. Various things cover it that make the doers of the women trafficking smoothly run their illegal business. The government so far has tried to do some kinds of preventions. However, still this activity goes wider and wider without being able to be stopped.

This is factual in which the women trafficking would be impossible to be prevented otherwise every line comprehensively commit to prevent it. So far, certain province in Indonesia, like West Java has tried to seriously overcome this problem. This province has cooperated with some other provinces like Riau and Riau Kepulauan to save the victims of women trafficking. Even, Wets Java's Government has law to prohibit the activity of women trafficking.

The activities of women trafficking is reflection of social reality. According to reference [6], what people do and say in their daily life reflects some aspects, such as honesty, responsibility, gentle attitude, and sincerity. However, in women trafficking, this reality is contradictory. There is no aspect explained above [7]. Even, since this situation occurs in West Sumatera in which they own philosophy that really supports these positive aspects [8][9]. Some external aspects make things change [10]. People tend to forget and ignore their tradition, culture, or social rules that lead the to the goodness.

### *2.2.Method*

The research is conducted in two districts in West Sumatera, they are Tanah Datar and Lima Puluh Kota. These two districts are selected based on the assumption that Tanah Datar is identical with the tight culture of Minangkabau. Many Minangkabau traditions and cultures are easily found there. Meanwhile, Lima Puluh Kota tends to be the target and the source of women trafficking.

The data are collected by observational method with note-taking and interviewing technique. Data are any utterances and information related to women trafficking. Besides, the documentation and related review of women trafficking are also used as the supporting information.

The analysis is done by referential, translational, and pragmatic identity method [11]. The concept related to women trafficking [2] and the way Minangkabau people communicate and behave [6] is used to analyze data. Each finding will be calculated to gain the percentage by simple statistical count (number of variable/ all data x 100%). The result of analysis is descriptively and narratively done.

## 3. Result andDiscussion

The are some depictions of social realities in the activities of women trafficking. They are (1) dishonesty; (2) money-oriented; (3) consumptive; and (4) carelessness. The

explanation of each will be described as follow.

### *3.1. Dishonesty*

Dishonesty is the opposite of honesty. Semantically, honesty means freedom from deceit or cheating. In contradictory, dishonesty is the situation of being deceit or cheating. In line with women trafficking, deceit or cheating becomes one variable depicted. This can be seen in utterance (1) below.

(1) ***Tanang se lah Etek! Ndak ka baa-baa gai anak gadih Etek ko do. Ambo jamin!***
'Just calm dowm, Aunty! Your daughter will be safe. I gurantee!'

The utterance is uttered by a young lady who is trying to persuade her villager-mate. This young lady called that hearer as *Etek* 'Aunty'. In Minangkabau society, *Etek* is term used to address some one who has relative relationship with us. In fact, the speaker has nothing do to with the hearer. This addressee is used to reduce the distance between the participants.

This young lady is persuading her *Etek* to let the daughter to go and work with her in the city. She offers the job to be the waitress in a café To assure this *Etek*, the young lady informed that the salary is big. Even sehe gurantedd that this Etek's daughter will be fine with her.

This utterance is only a kind of way in order the hearer may be imposed by the speaker. The speaker used some strategies. One of them is doing dishonesty. In the sense that, she did cheating, so the hearer may let her daughter go with that young lady.

### *3.2. Money-oriented*

Money-oriented is defined as the way to get money as much as possible in easy way and short time. This money-oriented attitude may commonly trap some girls to become the victims of women trafficking. For example is as being seen in utterance (2) below.

(2) ***Karajo ndak banyak do. Sabanta se nyo. Gajinyo gadang. Wak duduak-duduak se nyo malayani tamu. Kan sanang tu?***
'The work is not much. Only takes short time. The salary is big. We just seat and serve the guests. It is nice, isn't it?

The utterance (2) is uttered by a girl who has already worked in a town to another girl who is looking for the job. The girl (speaker) offers the hearer the job to be the guest servants in a café. According to the speaker, the work is not a lot since what the hearer supposed to do is only sitting and accompanying the guests who came to the café.

The speaker knows that the girl really wants the job that little work with a lot money. That is why she highlights her utterance by saying ***Gajinyo gadang*** and ***Kan sanang tu?*** This is reflected by the fact that the hearer accepted the job. What in her mind is that she will get much money by doing little thing.

### *3.2. Consumptive*

Consumptive means having tendency to be comsumption. This consumptive attitude has connotative meaning. In other words, those who are consumptively live are regarded negative. They sometimes consume something that they actually do not need yet. For example is as follow.

(3) ***Pokoknyo wak bisa mangganti hanphone iko, Kak. Handphone ko ndak canggih lai. Kawan-kawan wak ampia sakali 4 bulan baganti hpnyo. Wak ndak nio lo kalah do. Yang penting wak karajo, dapek pitih, ganti hp.***
'What I want is I can change this mobile phone with the new one. This mobile phone is not up dated any more. My friends mostly buy new mobile phone every 4 months. I do not want to get lose. The most important point is that I work, get money, and change the mobile

phone.'

This is an utterance of a girl, around 19 years old, to a lady who asked her to go the town to work. This young lady offers this girl a job as a waitress in a HP counter. She informed that the girl may buy any thing she wants from the salary that she is going to get as she works in that counter phone. This girl is eager to buy a new hand phone. In her understanding, she need to up date her phone periodically as her friends do. This girl does not think economically and wisely. Her phone is still good and new. However, since she is influenced by her environment of being consumptive, she then cannot help being consumptive either. Thus, the offer to work at the phone counter is finally accepted.

*3.2. Carelessness*

Careless means not taking care or thoughtless. Someone is categorized careless as s/he thinks carelessly. They do not thing fully before coming to the conclusion. Such kind of person is easily put in a trap of trafficking. S/he is persuaded by persuasive information. Without being checked and rechecked, this careless person directly believe in that. For example is as being shown in below utterances.

***Picayo se lah jo kakak! Ndak ka baa gai di amak tu do. Tando awak sayang ka urang gaek mah makonyo karajo. Cari pitih untuk panyanangan ati urang gaek tu!***

'Trust me! Your mother will permit you to go. This is the reflection of your love to your parents that you work. Get money to make your parents happy!'

A 30 years old lady persuaded a girl to go with her to the town. This lady informs that there is vacancy to be waitress in a clothes shop in the town. This shop does not consider the education level. The girl who only graduated elementary school is interested in that job. She thinks that with her very low education, getting job in the town as a waitress is miracle.

This girl fails to recheck her rights as the waitress, what she is going to do, and how much the salary will she get. What in her mind is going to the town, work, and get salary. Even, the lady adds that her mother will let her go since getting money may make her parents happy.

The reaction of the girl without rechecking the information is one reflection of being careless. The girl does not know yet the lady. She just met that lady in a mini market near her village. Her carelessness then puts her in problem since she was actually deceived by that lady.

These four reflection of social realities are some of many others. There are still some other possibilities of depiction of social realities in women trafficking activities. These four (1) dishonesty; (2) money-oriented; (3) consumptive; and (4) carelessness come up balanced. In the sense that, from around 45 data found, the occurrence of each reflection is almost the same.

However, dishonesty still comes with the highest percentage 33,3% (15 data) followed by carelessness 28, 9% (13 data); consumptive 22, 3% ( 10 data), and money-oriented 15,5% (7 data). Dishonesty appears as the highest since some external aspects like low-educational background and lack of knowledge about women trafficking makes some people are easily deceived by wrong information and wrong persuasion.

## Conclusion

Women trafficking is one of many realities found and be available in our live. This women trafficking flows like water as nobody seems disable to prevent. Ironically, the people may be unrealized that they are part of this women trafficking. Less of information about women trafficking makes many women becoming the victims. This must be handicapped. Otherwise, many women will fall into the hell of the world.

Having analyzed the data, the phenomena of women trafficking reflects four social realities. They are (1) dishonesty; (2) money-oriented; (3) consumptive; and (4) carelessness. These four realities may increase or decrease based on the willingness of government and society to prevent and save women from trafficking.

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**References**

[1] Trafficking and Fact Slavery Sheet. *21-30 Million People are Trapped in Slavery Around the Word Today*. 2012.

[2 Wheaton, Elizabeth M, Schauer, Edward J and Galli, Thomas V. *Economics of Human Trafficking. International Migration*, 48(4). 2010.

[3] Olujuwon, Tola. Combating Trafficking in Person: A Case Study of Nigeria. *European Journal of Scientific Research*, 24(1), 23-32. 2008.

[4] Muflichah, Siti dan Wintoro, Rahadi Wesi. 'Trafficking: SuatuStudi tentang Perdagangan Perempuan dari Aspek Sosial, Budaya, dan Ekonomi di Kabupaten Banyumas. *Jurnal Dinamika Hukum*. Vol. 9 No.1. pp. 10-16. 2009.

[5] Revita, Ike. *Research Report of Women Trafficking in West Java and West Sumatera*. Unpublished. Padang: Andalas University. 2015

[6] Oktavianus and Revita, Ike. *Kesantunan dalam Bahasa Minangkabau. Padang: Minangkabau Press. 2013*

[7] Murphy, Laura. *How Advocate Fighting Human Trafficking Can Overcome Divisions and Build Momentum.* SSN. US: Colombia Univ. Press. 2013.

[8] Errington, Frederick K. 1984. *Manner And Meaning in West Sumatera: The Social Context of Consciousness.* New York:Yale University

[9] Navis, A. A. *Alam Takambang Jadi Guru Adat dan Kebudayaan Minangkabau*. Jakarta: PT Grafiti Press . 1984

[10] Revita, Ike. 'Faktor-faktor Penanda Kesantunan dalam Tuturan Permintaan Berbahasa Minangkabau.' *Kongres Linguistik Nasional XII-2007*. Surakarta 3-6 September 2007

[11] Sudaryanto. *Metode Penelitian Bahasa Tahapan, Strategi, Metode dan Tekniknya*. Jakarta: Rajawali Press. 2013

# Efficiency of Marketing Distribution of Palm Oil in Sub District of Selesai Regency of Langkat

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## Abstract

Efficiency of marketing distribution is realized if the system provides the customer and stakeholders that involved in the distribution of product/service from the farmer as producer to the final consumer with satisfaction. Efficiency is the final goal will be achieved in a marketing system. The objective of this research is to identify the marketing distribution of palm oil in sub district of Selesai Regency of Langkat, to analyze the margin accepted by the marketing agency and to analyze the price accepted by the producer of palm oil in sub district of Selesai regency of Langkat. Sub district of Selesai is one of area in Regency of Langkat as producer of palm oil in Province of North Sumatera. Its location is very potential, reachable and has a strategic position that near to the palm oil factory of PT. Raja Tengah and PT. Amat Tani. In the marketing of their product, the farmer is helped by the small agent and big agent. The data is processed and analyzed by qualitative and quantitative method to study the efficiency of marketing distribution of palm oil. The research of qualitative analysis of efficiency of marketing distribution of palm oil through marketing agent and distributor, and the marketing function. The study of quantitative analysis of efficiency of marketing distribution of palm oil is described descriptively to describe the detail of marketing distribution, marketing function and any problems. While quantitative analysis was conducted by marketing margin approach, farmer's share, and profit to cost ratio. Based on analysis of marketing system it concluded that marketing distribution I is more efficient. This marketing distribution must be applied by farmer in sub district of Selesai. The other alternative that applied by farmer is to increase the quality of FFB, to build partnership to the palm oil factory, big and small agent, to maintain the quality of FFB and to up date the price information of FFB and the market development.

Keywords : Palm Oil, Marketing system, Efficiency of Marketing distribution

## 1. Introduction

Palm oil is one of the commodity crops that is important in economic activities in Indonesia. It is also one of Indonesia's export commodities are quite important as a producer of foreign exchange after oil and gas. Indonesia is the largest producer and the world's largest exporter of palm oil. North Sumatra Province is one of the areas that develop palm oil plantation commodities. Langkat is one area that is a center for palm oil development. It is supported by state of the climate and soil in accordance with the requirements grows for plantation crops. In general, community activities Langkat still oriented to the business of plantation crops and making commodities the main livelihood (Badan Pusat Statistik, 2014).

Efficiency of marketing channel in palm oil can be obtained through the analysis of the palm oil marketing system in Langkat Regency of North Sumatra Province, then evaluate how many marketing channels are on the distric are. Furthermore, analyzing the marketing margin, the farmer's share and calculate the benefit and cost ratio. High or low marketing margin is used to measure the efficiency of the marketing system (depending on the marketing functions are executed). The greater the marketing margin the more inefficient the marketing system. In the analysis of the farmer's share of the more expensive consumer pays the price offered by marketing agencies (agencies), then received by farmers will be less and less, because farmers sell agricultural commodity with a relatively low price. It showed a negative relationship between marketing margins with the share received by farmers (farmer's share). The larger margin caused relatively small revenue of the farmers. The magnitude of the ratio of benefits and costs is used to measure the efficiency of marketing channels. The higher the value of this ratio, indicating the benefits higher than the costs incurred. The result will recommend the most efficient channel to be implemented.

According Ratiza Alifa Asmarantaka (2013) in the Palm Oil Research Analysis of business administration at the village of Tanjung Jaya noted that the palm oil business administration at the village of Tanjung Jaya starting the farmer as a producer (manufacturer) to the processing mill, involving several marketing agencies. Agencies involved in the palm oil business administration at the study site are collectors and agent. Channel most used by respondent's farmers in marketing their palm oil is a marketing channel involving an intermediary agent and this is because farmers get bigger selling prices received by farmers so that profits are also relatively larger. Marketing functions performed by marketing institutions involved include exchange function, physical function, and the function of the facility has been running relatively well, but has not been implemented properly by several marketing agencies, especially farmers.

Marketing analysis results show that in each of the marketing agency is seen that the profit margin and the margin of the costs borne by each marketing agencies vary according to the marketing function has been carried out by each of brackish marketing agency. Value margin on channel I by 20.67 per cent and the value of margin on the line II of 34.67 percent. On the operational level of the second channel, the first channel is the most efficient channel. This is evident from the low marketing margin, the value of the farmer's share of the most high. However, the ratio of the value of $\pi$ / C this channel has the smallest value of 0.4. This channel is considered as an alternative efficient channel for achieving the welfare of farmers involved in this channel can be seen from the value of the margin and the farmer's share is generated and the sales volume of 71.85 tons, or about 69.7 percent of the total production of farmers.

Nurjannah Siregar (2014) conducted a study of Marketing of fresh fruit bunches of palm oil farmer in the village of Asam Jawa

Governmental District of Torgamba. Research results show that marketing margins are influenced by the farmer selling prices negatively and positively dummy marketing channels. The longer the marketing channels, the greater value of marketing margins. Channel marketing fresh fruit bunches (FFB) of palm oil contained in Asam Jawa village consists of 4 types, namely:

a. Marketing channels I: Manufacturers - Palm Oil Mill
b. Marketing channels II: Manufacturers - Wholesalers Small Gatherer – Palm oil mill
c. Marketing Channels III: Manufacturers - Wholesalers Great Gatherer – Palm oil mill
d. Channel Marketing IV: Manufacturers - Wholesalers Small Gatherer – Traders Great Gatherer - mills.

All marketing channels used by farmers have been efficient. Channels that are profitable for farmers is the marketing channel I, the merchant is marketing channels III and IV for the Palm oil mill is the channel. Palm oil mill is most beneficial for farmers and small traders are Palm oil mill PT. SSTL, and Palm oil mill with great profitable for the collector is PT.Milano.

Joldi Cristanto (2007) conducted a study of Marketing CPO (Crude Palm Oil) PT. IV Perkebunan Nusantara (PTPN-IV). Research results show that the marketing activities of CPO (Crude Palm Oil) applied by Joint Marketing Office (KPB) PTPN is open auction (tender) and long-term sales contract. Payment in cash held no later than fourteen (14) days after the date of the contract. Delivery of goods implemented not later than 15 days after the payment date. Quality quality (% Free Fatty Acid, moisture, and dirt), banking policy, the promotion, the port, and the competitors are the factors that affect the marketing activities of CPO (Crude Palm Oil) PT Perkebunan Nusantara IV. Determining the selling price of CPO (Crude Palm Oil) owned by PT Perkebunan Nusantara IV made by the Joint Marketing Office (KPB) is based on market mechanisms (prices prevailing in the market).

Share Margin marketing activities CPO (Crude Palm Oil) PT Perkebunan Nusantara IV to the domestic market, which is 96.72% while the share value of marketing margin CPO (Crude Palm Oil) PT Perkebunan Nusantara IV to markets abroad (exports), which amounted to 95, 31% and a value share of the total marketing margin CPO (Crude Palm Oil) PT Perkebunan Nusantara IV to markets abroad (exports) and the Interior (Domestic), ie 95.97%.

## 2. Materials and Methods

### *2.1 Sample*

One way of determining the amount of standard sample count is formulated by Slovin. Total numbers of samples take in this research are 70 people.

### *2.2 Data collection*

The data used in this study are primary data. The primary data obtained from direct observation (observation) and interview by using a list of questions presented on the questionnaire to palm oil farmers and traders as well as intermediary agent in Langkat. Direct observations were also conducted on the palm of marketing activities to determine the marketing channels and marketing agencies are involved in the marketing of palm oil groove.

### *2.3* Data analysis

The data is processed and analyzed by qualitative and quantitative methods to determine the efficiency of marketing channels. Research qualitative analysis of marketing channel efficiency of palm oil includes agencies and marketing channels, as well as the marketing function. Research qualitative analysis of marketing channel efficiency of palm oil is described descriptively to spell out all the details of marketing channels, through the marketing

margin approach, farmer share, and the benefits costs ratio.

## 3. Result and Discussion

Pattern of Marketing Channels FFB in District Selesai. According to Boyd, Walker and Larreche (2002), the marketing channel is the set of interdependent organizations involved in the process of making a product or service that is ready to be consumed or used by any consumer or industrial users. The main objective in designing a marketing channel is to find a combination of the most efficient intermediary for product specific market, channel minimizes distribution costs, but also to reach out and satisfy consumer targets.

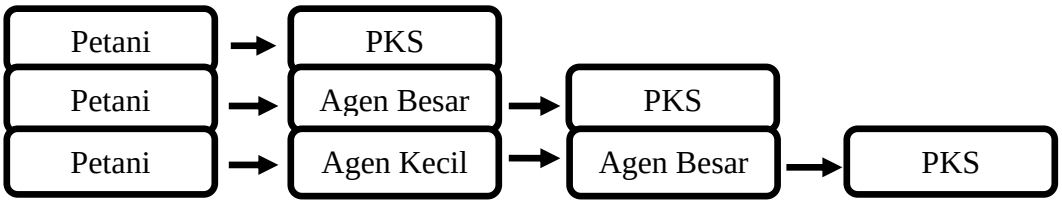


Figure 1. Schematic of Palm Oil Marketing Channel in the District Selesai

The study of 70 farmers of respondents, it can be seen that the pattern of marketing channels FFB through three pattern of marketing channel, ie the pattern of the first channel, the pattern of the second channel and the pattern of three channel. The pattern of the first channel TBS farmers sold directly to the mill. On the pattern of the second channel, TBS farmers sold to a large agency, and then directly transported to the mill. On the pattern of the third channel, TBS farmers sold to a small agent, then through a large agent transported to the mill. Scheme or pattern of palm oil marketing channels in the district overall Selesai can be seen in Figure 1.

*Marketing Margin Analysis*

Determining the level of efficiency of a system of marketing can be done through the analysis of marketing margins. Marketing margin is the sum of all costs of marketing/ marketing issued by marketing agencies and the fortunes that were taken in the commodity distribution activities from marketing agencies that one to the other marketing agencies. Marketing margin calculated in this study based on the pattern established marketing channels in the marketing activities of oil palm FFB in District Selesai. In this study, the marketing margin can be seen in every marketing channel.

*Analysis Farmer's Share*

Farmer's share is part of that received by farmers in a marketing activity and expressed as a percentage. Farmer's value share was obtained through a comparison of the price received by the farmer to the consumer level prices paid late. In this study, institutions that serve as the final consumer in the marketing activities of palm oil is the processing plant, using the level of the selling price of fresh fruit bunches when sold in the factory.

*Benefit and Cost Ratio*

Marketing costs are costs incurred by a number of marketing agencies in distributing fruit fresh bunches from the farm to the final consumer level expressed in rupiah per kilogram FFB. While the value of income is derived from the difference between marketing margins with marketing costs incurred in the implementation of marketing activities. The benefits costs ratio in marketing channels shows the amount of

benefits to be obtained per unit rupiah spent on marketing.

Table 2 Marketing Margin

| | Marketing Channels | | |
|---|---|---|---|
| | I | II | III |
| Price of farmers (Rp/Kg) | 1.690 | 1.445 | 1.410 |
| Price of Factory (Rp/Kg) | 1.690 | 1.690 | 1.690 |
| Margin (%) | 0% | 14,50% | 16,57% |

Source : Result of the research in Selesai Distric, 2016

Table 3. Farmer's Share based Pattern Marketing Channels Price-Level Marketing Channels

| Marketing Channels | Price of Farmers (Rp/kg) | Price of Factory (Rp/kg) | *Farmer's Share* (%) |
|---|---|---|---|
| Channel I | 1.690 | 1.690 | 100% |
| Channel II | 1.445 | 1.690 | 85,50% |
| Channel III | 1.410 | 1.690 | 83,43% |

Source : Result of the research in Selesai Distric, 2016

Table 4. Benefit and Cost Ratio

| Benefit Cost Ratio | Marketing Channels | | |
|---|---|---|---|
| | I | II | III |
| πi (Rp) | 1.240 | 135 | 150 |
| Ci (Rp) | 450 | 110 | 130 |
| Ratio πi/Ci | 2,75 | 1,23 | 1,15 |

Source : Result of the research in Selesai Distric, 2016

Table 5. Value of Marketing Efficiency on each - each Pattern Marketing Channels in Selesai District of Langkat Regency

| Efficiency Component | Indicator of Effeciency | Marketing Channel | | |
|---|---|---|---|---|
| | | I | II | III |
| Marketing margin (%) | Persentasi Terkecil | 0% | 14,50% | 16,57% |
| *Farmer's Share* (%) | Persentasi Terbesar | 100% | 85,50% | 83,43% |
| Benefit cost ratio | Lebih dari 1 | 2,75 | 1,23 | 1,15 |

Source : Result of the research in Selesai Distric, 2016

Efficiency is one of the objectives to be achieved in a marketing activity. The marketing system can be said to be done efficiently if the satisfaction of any person or institution involved in the implementation of the marketing system can be achieved. Parties or organizations not only consist of the actors involved in the process of distribution of the product, but rather to the final consumer level. It can be used as determinants of the efficiency of marketing activities including pattern of marketing channel is formed, the application of the marketing function in the distribution of products, the market structure, market behavior and marketing margins and the value of the farmer's share is formed.

## Conclusion

Based on the research that has been undertaken on the efficiency of marketing channels palm Done Langkat District of North Sumatra Province a number of conclusions including:

Channel marketing of fresh fruit bunches (FFB) of palm oil contained in Sub Done Langkat in North Sumatra Province consists of three kinds, namely:

a. Marketing Channels I: Farmers - Palm Oil Mill
b. Marketing Channels II: Farmers - Large Agent - Palm Oil Mill
c. Marketing Channels III: Farmers - Small Agents - Agents - Palm Oil Mill

Channel marketing is most widely used in the marketing of oil palm FFB is a marketing channel II as much as 72.85 percent.

The margin is the highest in distric Selesai is on the pattern of marketing channels III, 16.57 percent and low margins are the first marketing channel pattern is 0 percent for the first channel pattern of farmers do not use any intermediary. Marketing margin is affected by the farmer selling prices negatively and positively marketing channel patterns. The longer the marketing channels, the greater the value of marketing margins. The greater the marketing margin the more inefficient the marketing system.

The results of the analysis show that the marketing of each marketing institution is seen that the ratio of profits and the costs borne by each marketing agencies vary according to the marketing function that has been done by each marketing agencies. Value margin on the channel I is 0 percent and the value of the channel margin I is the most efficient channel. This is evident from the low marketing margin, the value of the farmer's share of the most high as 100 percent. However, the value of the ratio πi / Ci these channels have the greatest value is 2.75. This channel is considered as an alternative efficient channel for achieving the welfare of farmers involved in this channel can be seen from the value of the margin and the farmer's share generated.

## References

[BPS] Badan Pusat Statistik. 2014. Luas Tanaman dan Produksi Kelapa Sawit Tanaman Perkebunan Rakyat. [Internet]. [diunduh 2015 Desember 10]. Tersedia pada http://sumut.bps.go.id/frontend/index.php/linkTabelStatis/128.

Asmarantaka RA. 2013. Analisa Tataniaga Kelapa Sawit di Desa Tanjung Jaya Kecamatan Bangun Rejo Kabupaten Lampung Tengah Provinsi Lampung. Departemen Agribisnis Fakultas Ekonomi dan Manajemen. IPB. Bogor.

Boyd HW Jr, Walker OC Jr, Larreche Jean C. 2002. Manajemen Pemasaran Suatu Pendekatan Strategis Dengan Orientasi Global. Nurmawan I, penerjemah; Yatisumiharti, editor. Jakarta (ID): Erlangga. Terjemahan dari Marketing management, a strategic approach with global orientation.

Hanafie, Rita. 2010. Pengantar Ekonomi Pertanian. CV Andi Offset. Yogyakarta.

Cristanto, Joldi. 2007. Analisis Pemasaran CPO (Crude Oalm Oil) PT. Perkebunan Nusantara IV (PTPN-IV). Departemen Sosial Ekonomi Pertanian. Fakultas Pertanian. USU. Medan.

Kohl RL, Uhl JN. 2002. Marketing of Agricultural Products. London: New York an Coller Macmillan Publishing.

Kotler P, Susanto AB. 2000. Manajemen Pemasaran di Indonesia. Hermawan AA, penerjemah. Jakarta (ID): Salemba Empat.

Nalurita, Sari. 2008. Analisis Efisiensi Pemasaran Belimbing Dewa di Kecamatan Pancoran Mas Kota Depok Jawa Barat. Program Sarjana Ekstensi Manajemen Agribisnis. Fakultas Pertanian. IPB. Bogor.

Siregar, Nurjannah. 2014. Pemasaran Tandan Buah Segar Kelapa Sawit Petani Swadaya Di Desa Asam Jawa Kecamatan Torgamba Kabupaten Labuhanbatu Selatan. Fakultas Pertanian. Universitas Gadjah Mada. Yogyakarta.

# The occurrence of transitivity and suicidal motives on famous public figure`s suicide letters

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## Abstract

The aspects of transitivity in the suicide letters of famous public figures are related with forensic analysis on motives and mental process of the doers. The aims are: 1) to find the forms of transitivity (Process, Participant, and Circumstance) in suicide letters, 2) to draw the grand patterns occurred in those suicide letters, so that it can be used to investigate the motive of suicide in the perspective of forensic linguistics. The data were collected from 13 suicides letters written from famous public figures. The result of analysis of suicide letters are shown that firstly, the dominant process occurred was material process (46,4%) in which the description of actions which lead to suicide. It is followed by mental process (23,71%), relational attributive process (14,43%), existential process (9,3%), behavioural process (4,1%), and relational identifying process (2.3%). However, there is no existence of verbal process found. Secondly, in term of participant, the suicidal persons tend to use inanimate participants (41 variations) since it is influenced by the extra factors such as social environment. Thirdly, in term of circumstance, accompaniment circumstance is the most used (8 positions). In conclusion there is a tendency on those suicide letters to display the negative lexical registers that lead to the act of suicide.

*Keywords: Transitivity, Suicide Letter, Forensic Linguistics, Famous Public Figure, Motives of Suicide.*

## 1. Introduction

A suicide case becomes shocking news to all people who know because it belongs to criminal action. Especially, if the doer or actor of this case is one of the public figures, the treatment in investigating the case will be different and particular. The implication of suicide by famous public figures is ironically viewed since they who notably are role model for public yet ends their life tragically. The investigation is applied to reveal the factors behind. Observing and collecting information related to the suicide case are necessary to break the mystery of the death. Some of these public figures express it through their last words, known as suicide letters. This evident can be a tool to start identification of the motive. The concentration on the wordings system of the clauses on suicide letters actually depicts the messages of the writer. Furthermore, these clauses encode the function of their occurrence as the representation of writer`s urges before deciding suicide. In other words, the investigation in term of linguistic can be one of the ways to unveil the unseen motive.

The cases of suicide of famous public figures are often fraught with linguistic phenomenon and seen through their last letters. One of the most interesting elements while discerning this suicide letters is the linguistic register which is used by the suicidal person in displaying their feeling. The features are expressed mostly through direct statement such as “I leave this world” or “I may have a peace”. These examples are

merely some part of the flare up or emotional explosion of the writer. This phenomenon is attractive to be analyzed further because there is a big question of what are the influences of suicidal people tend to choose similar linguistic register in showing their agony, pain, or those dramatic feelings. Definitely, there is something hidden and peculiarity for this similarity, which lead to its function in representing their last messages and concealed motive. That is why assessment required.

The effort to asses or examine the language of suicide case is originally related to forensic linguistic. Olsson (2008: 128) states that the text types such as emergency calls, ransom demands, suicide letters, final death row statements, and confessions or denials by public figures belongs to forensic linguistic. Nevertheless, if this research merely follows the basic rule of forensic linguistic, the result is not complete. Since the main concern is about the function of clauses which express the meaning in the suicide text, this research possibly uses the systemic functional linguistics (SFL) as the blade to dig out the mystery behind it. Systemic functional linguistics proposed by Halliday (1994) provides the complete understanding about the function of clauses in the text of suicide letters which reflect messages of the suicidal person. There are three metafunction of language according to Halliday (1994:36);

Those three metafunction are reflected in every language. As Bloor and Bloor (2004: 2) said that "when people use language, their language acts produce or, or more technically, *construct* meaning". Thus, in order to find out the motive of suicide, it is possible to see it through form of writing (suicide letters). By looking of arrangement of words within suicide letters, the investigation will be leading to the revelation of writer`s intention in his or her messages. The representation of the messages produces many complexities to comprehend the motive.

The experiential metafunction basically concerns about the encoding of language as human experience in perceiving everything in this world (Bloor and Bloor 2004:107). This metafunction is reflected through the system of transitivity (Gerot and Wignell 1995: 52).

This transitivity becomes one of linguistic complexities which seem to be related to the suicidal motives. The scientific assumption is higher of transitivity so that effect on higher of the suicidal person`s intention. The transitivity itself refers to the reflection of all human`s activity where people can present their experiences fitting to the context of its situation. It is called by Halliday as the experiential function which is represented by transitivity (Collerson 1995: 12).

Through transitivity on which the reflection of experiences showed, the investigation of motives and hidden reason of suicide can be done. While examining the form of transitivity in the clauses, the features of suicide language can be observed by looking the wording systems which dominantly occur in these suicide letters. This language feature may be different if the user is a public figure. Their style of words in the letters automatically differs from other common suicidal person.

| **Metafunction (technical name)** | **Definition (kind of meaning)** | **Corresponding status of clause** |
|---|---|---|
| **Experiential/ Ideational** | Construing a model of experience | Clause as representation |
| **Interpersonal** | Enacting social relationships | Clause as exchange |
| **Textual** | Creating relevance to context | Clause as message |
| **Logical** | Construing logical relations | - |

Table 1: Three meta function of language

The background as famous public figure influences the way of communicating their grievance, problems, and disturbances within suicide letters. People normally consider that the flawless and perfect life always surround these famous people. There is no possibility of serious problems and hindrances to be faced by them. So the common matters perhaps are not presumed to be the motives of their suicide yet in fact it cannot be guaranteed.

In the way of observing the choices of word in suicide letters written by famous public figures, it can be seen what problems that they have, whether it is related to his personal life, carrier, or society. All of these matters are presented in his choices of words. Thus, the choices of word represent the feelings, thoughts, and actions of the suicidal person. Furthermore, the effort to analyze the transitivity and the choices of words in those suicide letters will lead to the motives of suicidal person.

## 2. Material and Methods

This research basically refers to qualitative approach in term of descriptive analysis. As Neuman (1997: 327 - 328) reports that when data are in the form of words, sentences, and paragraphs rather than number, it refers to qualitative research. It is relevant to the data of this research based on words and clauses of suicide letter. Moreover, he also presents "qualitative data are empirical which involve documenting real events, recording what people say (with words, gestures, and tone), observing specific behaviors, studying written documents or examining visual images". Suicide letters itself are taken from the real event from suicide cases of famous public figures.

In the process of collecting data, the non participant observational method is used since there is no interaction happen between researcher and the object of data in the context of creating linguistics phenomenon (Sudaryanto 1988: 2). In order to check and to verify the validation of this research, I apply the method of triangulation. As Stake (1995) states the protocols that are used to ensure accuracy and alternative explanations are called triangulation.

The need for triangulation arises from the ethical need to confirm the validity of the processes. This method is divided into several types; triangulation of data sources, investigator, theory, methodology, and environment (Guion, Diehl and McDonald 2011). Here, I specially conduct the triangulation to verify the source of data. I attempt to collect and compare several different websites in internet containing similar suicide letters. In addition by analyzing and comparing other related documents, articles, and archival records of suicide case of famous public figures, the source of data can be counted as the right one. The result is 13 data were chosen.

In the process of analyzing the data, firstly the analysis is begun by providing the scientific assumption which has been mentioned in the previous explanation. The transitivity may leads to the degree of intention in committing suicide. The representation of this intention or ideas actually is depicted through the arrangement of clauses of the letters. Starting from this, the messages are reflected through the form of description of processes in certain circumstances done by the participant (the suicidal person).

After that, I begin with sorting and distributing each elements of transitivity for each clauses of a suicide letters. Then I endeavor to seek out the mostly process occurred. As the result, the dominance of process will be seen and counted by the descriptive statistic. Sarwono says that (2006: 138) descriptive statistic is a transformation of raw data into the easier to comprehend and interpret the meaning of data or number presented. The goal is to figure out the observational answers and results including distributional of frequency, percentage, and

mean. Yet, the intention is pointed to the distributional frequency since the purpose merely looks for the dominance. To account and accumulate the amount of dominance process in the clauses, the descriptive statistic is employed.

Having found and got the set of transitivity and its dominance, the context of situation and social are connected into the arrangement of the words in the suicide letters. Each of processes and the things embedded to it will be explained by the situational context within the text. In addition, finding the form of lexical register which mostly applied in these suicide letters is also one of the focus of this research. Starting to this step, I try to draw the style of language which commonly is used by this community, suicidal persons. Here, by looking the most occurrence lexical used, it can contribute to determine the genre of the text.

## 3. Result and Discussion

Early, Halliday (1994:106) has remarked "The transitivity system construe the world of experience into a manageable set of process types". What this statement mean is to strengthen a notion that text is the representation of our experience. In other words, what is going on that is illuminated by actions that require process in it. There are there elements; they are circumstances, processes, and participants.

Those elements will expose the hidden message of the suicide letters written by famous public figures. By showing each role of them, it assists the investigation of motives through the arrangement of the words of suicide letters.

### a. Analysis on the type of process

According to Halliday there are six types of process however there is an addition process provided by Gerot and Wignell (Gerot and Wignell 1995:54);

1. Material Process is process of material doing. This process obligatory have a doing (process) and a doer (participant). The participant who does something is the *Actor* and the process to be achieved is *Goal*.
2. Mental Process is process of sensing; feeling, thinking, perceiving through the five senses). Thus the participant roles are *Senser* and *Phenomenon*.
3. Behavioral Process is process of psychological behavior, like breathing, snoring, looking, and etc. the obligatory Participant is the *Behaver*
4. Verbal Process is process of saying, or more accurately, of symbolically signaling. The obligatory participant is *Sayer* (signal source). There are three other Participants that may be incumbent upon Verbal processes; *receiver* (the one to whom the verbalization is addressed), *target* (one acted upon verbally such as insulted, complimented, etc), *range/verbiage* (a name for the verbalization itself).
5. Relational Process involves states of being (including having). There are two parts; *identifying process* that has obligatory participant roles are *Token* (the sign, name, form, holder, occupant) and *Value* (the referent, function, and status), *Attributive Process* that has obligatory participant roles are *Carrier* and *Attributive*.
6. Existential Process is process of existence. It is expressed by verbs of existing; 'be', 'exist', 'arise', and the *Existent* can be a phenomenon of any kind.
7. Meteorological Process is related to process of weathering that is indicated by pronoun 'it' that has no representational function, but does provide a subject.

This analysis is started by describing the form of transitivity. Then, the motives of suicide can be seen in the arrangement of its transitivity. This following example will show about the process of how the transitivity reflects the motives of suicide.

The Suicide Letters written by George Sanders:

> *Dear World, I am leaving you (1) because I am bored (2). I feel (3) I have lived long enough (4). I am leaving you with your worries in this sweet cesspool (5) - good luck (6).* (George Sanders, British actor, d. April 25, 1972)

This suicide letter was written by George Sanders, a British actor. From the six clauses involved, there are three processes of material and three for mental. Each of them has certain roles in conveying and communicating its meaning. These two processes share balance position in the text. The material process are found in clauses (1.1, 1.4, 1.5) meanwhile the mental process exists in (1.2, 1.3, 1.6) clauses.

The description of all the sentences above use direct statement which indicate the writer does not have a hesitance about his intention. From the first clause (1.1), it can be seen the process of material in clause "I am leaving" which seems there is a mental forces to remark this idea to be real. When it is seen by its appearance, certainly it is categorized as material. The writer intends to go and leave something. The implication of this clause is not merely a plain meaning such a process of leaving in temporary way, yet it refers to end his life and leave all of things in this world forever. So that in other words, the mental process strongly underlies the material process. It is a final decision of this writer in determining his life. The verb "am leaving" which refers to the world gives a sign that the writer has long enough to think his plan to die.

Here, this statement is repeated twice in the letter (1.1 and 1.5); therefore the self confidence of the writer to commit suicide is not bargainable. It is convinced by the mental process in the second clause and the third one. The phrase "I am bored" in second clause (1.2) implies the hollowness of his feeling since he wrote the boredom. In addition in the third clause (1.3), the phrase "I feel" and fourth one (1.4) "I have lived" explain that he feels the limit of thinking and expresses the desperation by saying he has enough lived in this world. There is no passion to be achieved anymore and it is a culmination of all experiences which he has encountered in life. Those problems urge him too much then he cannot bear with that anymore. He thinks them as scrapheap or dumps which where deserve to be thrown away. Nevertheless, there is a satire (1.5) in his expression by presenting it as "sweet cesspool" (sweet toilet).

| | | | | | |
|---|---|---|---|---|---|
| 1.1 | Dear world, | I | am leaving | you | |
| | | Actor | Pro: Mat | Goal | |
| 1.2 | Because | I | | am bored | |
| | | Senser | | Pro: Men:Aff | |
| 1.3 | | I | | feel, | |
| | | Senser | | Pro: Men:Aff | |
| 1.4 | | I | have lived | long enough | |
| | | Actor | Pro: Mat | Cir: Time | |
| 1.5 | I | am leaving | you, | with your worries | in this sweet cesspool |
| | Actor | Pro: Mat | Goal | Cir:Accomp | Cir: Place |
| 1.6 | Good luck → | ( I | wish | you) | good luck |
| | | Senser | Pro: Men:aff | Phenomenon | Phenomenon |

| Material | Mental | Relational | | Verbal | Behavioural | Existential |
|---|---|---|---|---|---|---|
| | | Attributive | Identifying | | | |
| **1.1** | 1.2 | 2.1 | 2.3 | _ | 10.12 | 8.5 |
| **1.4** | 1.3 | 5.1 | 10.10 | _ | 11.1b | 10.2 |
| **1.5** | 1.6 | 6.3 | _ | _ | 11.4 | 10.3 |
| **3.2** | 2.2 | 10.1 | _ | _ | 11.18 | 10.4 |
| **4.1** | 3.1 | 10.7 | _ | _ | _ | 10.5 |
| **4.2** | 6.2 | 10.8 | _ | _ | _ | 10.6 |
| **5.2** | 7.1 | 10.9 | _ | _ | _ | 10.11 |
| **6.1** | 9.1 | 11.17a | _ | _ | _ | 12.2 |
| **6.4** | 9.2a | 11.21 | _ | _ | _ | 12.3 |
| **6.5a** | 9.3a | 11.23 | _ | _ | _ | _ |
| **6.5b** | 11.1a | 12.4a | _ | _ | _ | _ |
| **7.2** | 11.2a | 12.5 | _ | _ | _ | _ |
| **7.3** | 11.5 | 13.1 | | | | |
| **8.1** | 11.9a | 13.3a | _ | _ | _ | _ |
| **8.2** | 11.9b | _ | _ | _ | _ | _ |
| **8.3** | 11.12a | _ | _ | _ | _ | _ |
| **8.4** | 11.14b | _ | _ | _ | _ | _ |
| **9.2b** | 11.15a | _ | _ | _ | _ | _ |
| **10.14b** | _ | _ | _ | _ | _ | _ |
| **9.3b** | 11.17b | _ | _ | _ | _ | _ |
| **9.4a** | 11.19b | _ | _ | _ | _ | _ |
| **9.4b** | 11.25a | _ | _ | _ | _ | _ |
| **10.13** | 11.25b | _ | _ | _ | _ | _ |
| **10.14a** | 13.2 | _ | _ | _ | _ | _ |
| **11.2b** | _ | _ | _ | _ | _ | _ |
| **11.3** | _ | _ | _ | _ | _ | _ |
| **11.6a** | _ | _ | _ | _ | _ | _ |
| **11.6b** | _ | _ | _ | _ | _ | _ |
| **11.7** | _ | _ | _ | _ | _ | _ |
| **11.8a** | _ | _ | _ | _ | _ | _ |
| **11.8b** | _ | _ | _ | _ | _ | _ |
| **11.10** | _ | _ | _ | _ | _ | _ |
| **11.11** | _ | _ | _ | _ | _ | _ |
| **11.12b** | _ | _ | _ | _ | _ | _ |
| **11.13** | _ | _ | _ | _ | _ | _ |
| **11.14a** | _ | _ | _ | _ | _ | _ |
| **11.15b** | _ | _ | _ | _ | _ | _ |
| **11.16** | _ | _ | _ | _ | _ | _ |
| **11.19a** | _ | _ | _ | _ | _ | _ |
| **11.20** | _ | _ | _ | _ | _ | _ |
| **11.22** | _ | _ | _ | _ | _ | _ |
| **11.24** | _ | _ | _ | _ | _ | _ |
| **12.1** | _ | _ | _ | _ | _ | _ |
| **12.4b** | _ | _ | _ | _ | _ | _ |
| **13.3b** | _ | _ | _ | _ | _ | _ |
| | | | **Total** | | | |
| **45** | 23 | 14 | 2 | 0 | 4 | 9 |

**Table 2: Summary The Types of Process in Famous Public Figure`s Suicide Letters**

In this place, he leaves all worries of the world which are illuminated by the dirtiness. In other words, he feels weary of all the dirtiness which exists in this world. There are complex problems so that he cannot endure it anymore. He finally signs off to this world. At

final statement, he wishes for those who still stand up in this world to be good and luck in countering that dirtiness. The mental process "wish" in clause (1.6) figures out the concern about peoples around him before committing suicide.

Furthermore, this is the summary of all processes in the different 13 suicide letters. It consists of 97 processes. The implementation can be seen in table 2. It is seen that the material process is the most dominant among others. It takes the first place with 45 clauses and is followed by the mental process with 23 clauses. Then it is consecutively followed by relational attributive is 14 clauses, existential is 9 clauses, behavioural is 4 clauses, and relational identifying is 2 clauses. Furthermore, it is described in Diagram 1.

The diagram above is obviously described that the first position belongs to material process (46,4%). The second and third process orderly are involved by mental process (23,7%) and relational attributive process (14,4%). Then, in the three remaining process is positioned in the fourth place by existential process (9,3%), the fifth place is behavioural process (4,1%) and the last position is hosted by relational identifying process (2,1%).

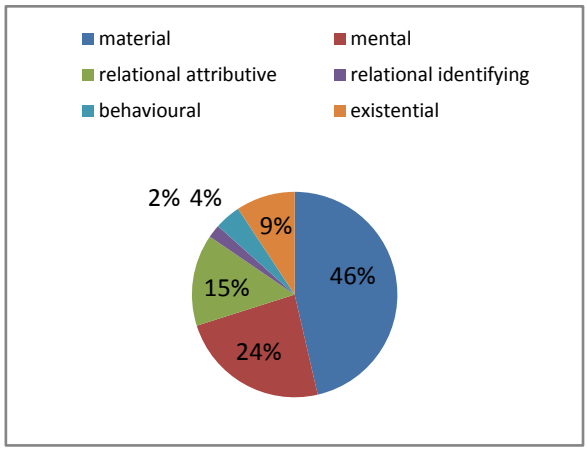


Diagram 1. Types of Process in Famous Public Figure`s Suicide Letters

Looking the types of process holistically, the verbal process never appears in those suicide letters. The verbal process itself refers to the process of saying or symbolically signaling. It is started by the statement uttered by a speaker and it is repeated again by the hearer. The probability process of saying and restating the statement seems rarely happened in the texts of suicide letters. The process of giving and receiving statement of course is involved by at least two persons. However, in term of the text of suicide letters, all statement derives from the suicidal person himself. Since suicide letter is the representation of someone feeling. The commitment of committing suicide never comes from someone else. It is coming from oneself. Thus, it does not need any comments, statements, and opinions from others. It is purely derived from the impending contemplation of someone since it is not simple and easy decision.

In contrary, based on the description of the statistic above, it can be seen that material process and mental process are the most dominantly used in conveying the feeling and delivering the intention of the writers of those suicide letters. It happens because the material process is the reflection of all action to do suicide. This is the description all experiences and the goal to commit suicide. Of course it happens, because the only intention is to commit suicide. In addition, mental process underlies the realization of this material process. In other words, all action that stated or uttered in suicide letter are triggered by the impulse, pressure, and encouragement of feelings. Feelings of pain, hatred, deep boredom, and depression about all problems which they encounter in running their life are the mental process which initiates the idea of committing suicide appeared.

| No | Animate | Inanimate |
|---|---|---|
| **1** | I | The future |
| **2** | You | Old age |
| **3** | People | Illness |
| **4** | God | Pain, This agonizing pain |
| **5** | We | Peace |
| **6** | Me | The only way |
| **7** | My baby | This |
| **8** | Our baby`s | Their own live, my life away, spoiling your life |
| **9** | Him | This world |
| **10** | Anyone | The heart |
| **11** | Two people | My work |
| **12** | Everybody | The great adventure |
| **13** | Anybody | It |
| **14** | one | My body |
| **15** | | The impending blindness |
| **16** | | My soul |
| **17** | | Fun, no more fun, no fun for anybody |
| **18** | | A death pact, a quick and easy death, an avoidable and imminent death |
| **19** | | My part of the deal |
| **20** | | Football season |
| **21** | | Over |
| **22** | | No more games |
| **23** | | No more bombs |
| **24** | | No more walking |
| **25** | | No more swimming |
| **26** | | The simplest of human right |
| **27** | | 17 years past 50 |
| **28** | | 17 |
| **No.** | **Animate** | **Inanimate** |
| **29** | | Boring |
| **30** | | Bitchy |
| **31** | | Greedy |
| **32** | | Certain |
| **33** | | Mad |
| **34** | | Voices |
| **35** | | What seems the best thing, Everything, anything |
| **36** | | The greatest possible happiness, all the happiness of my life |
| **37** | | This terrible disease |
| **38** | | The certainty of your goodness |
| **39** | | My decision |
| **40** | | All usefulness |
| **41** | | Lead |
| **Tot.** | 14 | 41 |

**Table 3: Types of Participant in Famous Public Figure`s Suicide Letters**

b. Analysis on the type of participant

This is the description of the types of participants in the suicide letter written by famous public figures. Among the 97 clauses, there are two kinds of participant with different variation of choices.

The above table shows that the inanimate participant dominates with 41 variations among 97 clauses of suicide letters written by famous public figures. Meanwhile the animate participant only has 14 variations. This means that the writers of suicide letter tend to use and apply the inanimate participant to represent their feeling or the messages. The number of inanimate participant itself occurs basically because of

the environmental and social influences. They derive from the external factor or extra linguistic factor which are more than the influence of internal or intra linguistic factor. This is normal, since the language and the environment are related to each other. The clauses exist because of the reflection of human activity, natural phenomenon, and social environment. All of them unite together and give the biggest impact on the way of people thinking, perceiving, and doing something. It happens because of the encouragement and pressure of the all things around human so that the use of inanimate object dominates.

In other words, all extra linguistic factors such as pain, deep boredom, illness, hatred, problems and any kinds of things that related to depression trigger the occurrence of suicidal motive in famous public figures. Thus, the inanimate participant frequently used in delivering and expressing the feeling and problems of suicidal person which are categorized as the last message before committing suicide. That reasons are called the motives.

**c. Analysis on the type of circumstance**

This is the types of circumstance which are applied in among 13 suicide letters shown in table 4. From the table, the dominant circumstance goes to accompaniment with 8 positions. It is followed by the time circumstance and place circumstance with 6 positions per each of them. Meanwhile the application of manner circumstance which is divided into two types; they are manner-quality with 4 positions and manner- means with 1 position follow.

The most applied circumstance is accompaniment which exists 8 times. It is followed by time and place which are sharing the point in 6 times each of them. In addition the manner of quality gets 4 times and manner of means has one time.

The reason behind the dominant occurrence of accompaniment is because of the strong attraction of using the accompaniment to complete the description situation that is experienced by the suicidal person. This feature assists someone more freely to express their feeling in any kind of situation and condition.

**d. Lexical Register**

Reviewing all words which regularly used in the famous public figure`s suicide letter, there some particular words rise. The words and phrases which lead to negative perspective are used often. Those words basically are the reflection of problems, forces, and polemics encountered by the famous public figures. The words which reflect pain, hatred, sadness, decision, deep boredom, and depression illuminate mostly in the suicide letter written by famous public figures. It is seen in table 5. From the table, it is seen that the lexical register which refer to negative sense shows the refusal of all conditions which are faced by the suicidal person. The pain, hatred, sadness, deep boredom, and decision orientate to self destruction. This self destruction leads to kill oneself. This decision is made as the effect of the unsolved problems and psychological problems so that someone wants to leave it behind by leaving this world or in other words to commit suicide.

**Conclusion**

Based on the analysis of transitivity and motives of suicidal person of famous public figure`s in 13 suicide letters and the finding of grand pattern of these suicide letters, it can be concluded that all suicide letters tend to use the material process (46,4%) and mental process (23,71%) out of 97 clauses in expressing the feeling of suicidal person before committing suicide. Then, the relational attributive process occupied the third position (14,43%). The remaining processes are existential process (9,3%),

behavioural process (4,1%) and relational identifying (2,3%).

**Table 4: Types of Circumstance in Famous Public Figure`s Suicide Letters**

| Circumstance | Time | Place | Manner | | Accompaniment | Cause | Role | Matter |
|---|---|---|---|---|---|---|---|---|
| | | | means | Quality | | | | |
| **Frequency** | 6 | 6 | 1 | 4 | 8 | - | - | - |

**Table 5: The List of Frequent Lexical Register in Suicide Letter of Famous Public Figures**

| Pain | Hatred | Sadness | Decision | Deep Boredom | Depression |
|---|---|---|---|---|---|
| **Illness** | Greedy | No more games | Must end | Boring | To make a great adventure |
| **This agonizing pain** | Bitchy | No more bombs | The only way | Bored | My part of the deal |
| **The impending blindness** | Mad | No more walking | What seems the best thing | | My life away |
| **Terrible disease** | | No more fun | A quick and easy death | | Unavoidable and imminent death |
| | | No more swimming | A death pact | | |
| | | No fun for anybody | | | |

Most of the material processes were involved by very strong decision of committing suicide. It can be inferred from those who put decision statement in the initial position in his suicide letter by stating to leave this world. Then, the actor of suicide defined their reason and the background of committing suicide. Moreover, in composing the letter, the suicidal person usually had serious contemplation on his action. This is not an instant decision. This intended action is underlined by the mental process. The pressures, urges, and the problems of a suicidal person lead him to depression. It is become worse and then decides to give up. Therefore, in order to escape from his depression, he commits suicide.

However, the verbal process never occurs in the 13 suicide letters, because verbal process requires the process of giving and receiving statement. Yet, in the text of suicide letters it never happens. The suicide letter is result of someone contemplation. It comes from the soul of the suicidal person himself. There is no pressure from someone else. Then, in term of types of participant, those suicidal persons prefer to use the inanimate variation (41 variations) rather than animate variation (14 variations). The inanimate variations itself refer to the extra linguistic factors which influence the appearance of internal linguistic factors. The inanimate variations are mostly related to social and environmental problems encountered by suicidal person. Then in the form of circumstance, accompaniment (8 positions) is the most applied in those suicide letters. The reason behind the dominant occurrence of accompaniment is because of the strong attraction of using the accompaniment to complete the description situation that is experienced by the suicidal person. This feature assists someone more freely to express their feeling in any kind of situation and condition.

There are also many kinds of negative words which reflect “pain”, “hatred”, “mad”, “sadness”, “terrible disease”, “deep boredom”, and “final decision”. Those words have orientation toward the self destruction. Most of them failed to fight and survive in this world. There is no hope, desires, efforts, and dreams to be the shield in preventing suicide.

From the exposition above it can be summarized that the motive of suicide of

famous public figures are mostly because of the pressure of social environment to be perfect, the boredom of the current condition such as impending illness and drugs addiction, and refusal on the world regulation. These factors cause the deep depression and the decision of leaving this world.

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## References

Bloor, T., and Bloor, M. 2004. The Functional Analysis of English: A Hallidayan Approach 2nd ed. Great Britain. Oxford University Press Inc.

Collerson, J. 1994. English Grammar: A Functional Approach. Australia. Primary English Teaching Association.

Eggins, S. 2004. An Introduction to Systemic Functional Linguistics. 2nd ed. New York. Continuum International Publishing Group.

Gerot, L., and Wignell, P. 1995. Making Sense of Functional Grammar. Australia. Gerd Stabler.

Guion, L. A., Diehl, D. C., and McDonald. 2011. Triangulasi: Establishing the Validity of Qualitative Studies. FCS6014. Department of Family, Youth and Community Sciences, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Retrived 30th April 2013 from http://edis.ifas.ufl.edu.

Halliday, M. A. K. 1994. An Introduction to functional grammar. Second Edition. London: Arnold.

Halliday, M. A. K., and Hasan, R. 1985. Language, context, and text: aspects of language in a social-semiotic perspective. Oxford: Oxford University Press.

Neuman, W. Lawrence. 1997. Social research methods: Qualitative and quantitative approaches 3rd ed. Unites States of America. Allyn and Bacon.

Olsson, J. 2008. Forensic linguistic 2nd ed. Great Britain: Cromwell Press.

Sarwono, J. 2006. Metode penelitian kuantitatif dan kualitatif. Bandung: Graha Ilmu.

Stake, R. 1995. The art of case research. Thousand Oaks, CA: Sage Publications.

Sudaryanto. 1993. Metode dan aneka teknik analisis bahasa. Yogyakarta: Duta Wacana University Press.

# An analysis of Marketing Efficiency of Sapodilla in Nagari Sumpur sub district of Tanah Datar, West Sumatra

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## Abstract

Nagari Sumpur is the biggest producer of sapodilla in Tanah Datar sub district even in West Sumatra. However, economically this agricultural commodity does not provide a high advantage for the farmers in this area. There is a big gap between the farmers' price and the price paid by the final consumers. The research finds that the highest market efficiency is in direct market from farmers to small wholesalers. However there are only 5 percent of farmers in this pattern of market channel, while the rest of farmers sell their product to pickers who will sell it to big and small wholesalers. Most of farmers prefer to sell their product to pickers due to the practically reason and they don't need to spent any cost on it. Apparently the farmers lack of effort in managing and marketing their product. This is might be because psychologically they realize that they did not plant sapodilla by themselves but own it by heritage. Furthermore, farmers have a weak bargaining position and price is set by wholesalers in outside of region. Since 80 percent of sapodilla from Nagari Sumpur is sold to other provinces, the price is also influenced by sapodilla production of other regions. Additionally, the farmers do not have any options other than sell their product on any price because they don't have other alternatives. Accordingly, there should be an effort to create value added of sapodilla, so that the farmers have other alternatives toward their product.

Keywords: marketing, efficiency, SCP, farmers, price

## 1. Introduction

Sapodilla is a long-lived tree that is native to Guatemala (Central America), Mexico and the West Indies. In recent years, sapodilla is spreading throughout tropical countries worldwide such as India, Thailand, Malaysia, Indonesia, Cambodia, Bangladesh and Mexico. In Indonesia, sapodilla has long been known and widely planted in regions with a height of 1200 m above sea level, such as in Java and Madura (Rismunandar, 1983).

In Indonesia, total production of sapodilla is still relatively low compared to the production of other fruits. This is because in Indonesia most of sapodilla is not cultivated commercially, but is only planted in the yard. Although sapodilla production in Indonesia is still low, but this plant has spread almost throughout the territory of Indonesia. According to data released by the Central Bureau of Statistics in the period of 2009-2013, the main producers of sapodilla in Indonesia are West Java, Lampung and West Sumatra with a total production of 93.293 ton, 67.649 ton and 56.514 ton respectively.

Sapodilla is usually consumed as a fresh fruit. Sapodilla contains fat, protein, vitamins A, B, and C, iron, calcium, phosphorus and other minerals. Sapodilla plants are not only beneficial in terms of consumption trees, but also as reforestation trees in arid and critical lands, and also as a raw material for the manufacture of household furnishings (Prihatman, 2000).

One of the provinces with the largest production of sapodilla is West Sumatra. Sapodilla is one of the major fruit commodities in West Sumatra. It can be seen from the LQ index (Location Quotient) of this commodity which is greater than one (1.72) that can be categorized as high category. If a commodity has LQ index > 1; it means the commodity has a comparative advantage and can supply the demands of other regions. When the LQ index = 1, it means the region does not have a comparative advantage and its production is only able to fulfill the local needs. However, if the LQ index <1; it means that the commodity cannot fulfill the local needs, so that the region needs to supply from the other regions (Dipti, 1997).

Moreover the biggest producer of sapodilla in West Sumatra is Tanah Datar District with a total harvested area of 342.34 hectares. Based on several indicators such as LQ, the level of suitability agro technology, financial and economic feasibility, ability to create value added and employment, Tanah Datar is the center production of sapodilla in West Sumatra. Furthermore, the area with the largest production of sapodilla in Tanah Datar district is sub-district of South Batipuh, precisely in Nagari Sumpur. Sapodilla production in this region reached 7596.60 tons and harvested area of 201.60 Ha (Badan Litbang Pertanian, 2012).

The variety of Sapodilla cultivated in Nagari Sumpur is variety manila (*Manilkara zapota*), which had been cultivated since the Dutch era and continuously maintained until recently. Thus the age of sapodilla plants in this area are already over 75 years and still keep producing till nowadays. Sapodilla from Nagari Sumpur is famous for its sweet taste and is already marketed to provinces outside of Sumatra Island. Sapodilla has become one of the main sources of income in Nagari Sumpur. This plant has become mandatory that should be planted by the villager in the yard of their home. Besides planted in the yard, sapodilla has also been planted in the garden located in the hills.

One aspect of marketing that need to be considered in order to improve the flow of goods from producers to consumers is the marketing efficiency. Marketing efficiency shows the prices differences received by farmers and paid by the final consumer and also the feasibility of income received by farmers and other marketing agencies involved in the marketing activities.

Furthermore, the market of sapodilla of Nagari Sumpur is not only in West Sumatra but also other provinces. Based on previous research, the market of sapodilla from Nagari Sumpur has reached not only provinces in Sumatra but also provinces outside Sumatra such as Jakarta and Bandung. However the farmers tend to get the smallest portion of marketing share. Recently the farmers sell sapodilla at price of Rp 3.300/ kg, while the price of Sapodilla at consumer level in West Sumatra is Rp 12.000- Rp 15.000 per kg. The farmers have a weak bargaining position, due to they don't have other alternatives rather than sell their product freshly at any price. There are several patterns of marketing channels of sapodilla Nagari Sumpur that involves several marketing institutions which are farmers, local assemblers, wholesalers and retailers. In this case appears that the farmers get a fairly small part in the marketing channel.

## 2. Material and Methods

This study was designed as a survey. In general, the method used in this study was a combination of qualitative and quantitative method. Qualitative method carried out firstly to find specifically the hypotheses of relationships between variables which will be analyzed quantitatively.

Methodologically, in order to identify and provide a clearer picture of the marketing channels of sapodilla from farmers to end consumers in Nagri Sumpur will be carried out using descriptive qualitative method. The

data were collected from marketing agencies that play a role in the marketing chain of sapodilla. Furthermore marketing efficiency of sapodilla analyzed using SCP model (structure, conduct and performance).

## 3. Result and Discussion

### 3.1 Overview of Nagari Sumpur and Sapodilla

Nagari Sumpur located in South Batipuh subdistrict, Tanah Datar, West Sumatra Province, Indonesia. Nagari Sumpur is located near Danau Singkarak and has a hilly topography. This village (nagari) consists of five sub villages (jorong) which are Jorong Batu Baraguang, Jorong Nagari, Jorong Kubu Gadang, Jorong Subarang Aie Taman and Jorong Suduik. The village with a total area of 7,34 $km^2$ has a population of 2.246 inhabitants.

The community of Nagari Sumpur generally depends on sapodilla as resource of their income. The other income resources are bilih fish and paddy field. Sapodilla has become the main pillar of the economy for local residents since it had been cultivated by Dutch colonial decades ago. Initially Sapodilla was just planted in the yard of their houses, but today it is also cultivated in hills.
Historically, in Dutch colonial era, the Dutch want to increase production of spices such as nutmeg and clove by extending the cultivation area to Guguak Malalo. Since in order to get to Guguak Malalo must go through Nagari Sumpur, then Dutch colonial persuaded the residents of Nagari Sumpur with Sapodilla. Back then the Dutch colonial cultivated sapodilla for residents in Nagari Sumpur and since then the resident keep maintaining the sapodilla. Additionally, the current sapodilla owned by farmers in Nagari Sumpur is the sapodilla cultivated decades ago. Since then, Sapodilla has become the important plant for the society of Nagari sumpur. Formerly, there was a regulation in Nagari Sumpur that a man required planting at least 5 sapodilla trees before married. This regulation indicated the importance of sapodilla for the society of Nagari Sumpur either economically or customarily. Recently sapodilla is not only cultivated in Sumpur but also in neighborhoods such as Malalo, Batu Taba and Padang Laweh. In 1994 the ministry of agriculture assigned Sumpur's sapodilla as a superior variety of sapodilla (Raesi, 2013). Afterwards in 2004 the first sapodilla farmer group was established in Nagari Sumpur namely Kelompok Tani Sawah Tanjung and in 2008 one more sapodilla farmer group was also established namely Kelompok Tani Tunas Baru.

### 3.2 The Market Structure of Sapodilla

Generally natives Nagari sumpur have land planted with Sapodilla, thus there are many sapodilla producers in this area. The only difference is the number of Sapodilla trees they have. In 2014 Department of agriculture of Tanah Datar registered sapodilla field in order to prepare the farmers for Asean Free Trade Area.

Furthermore there are five buyers of Sapodilla in Nagari Sumpur namely end consumers, pickers, big wholesalers, small wholesalers and retailers. The research finds that most of the farmers sell their product to pickers due to the practically reason and they don't need to spent any cost on marketing. Apparently the farmers lack of effort in managing and marketing their product. This is might be because psychologically they realize that they did not plant sapodilla by themselves but own it by heritage. Approximately only 5 percent of farmers sell sapodilla directly to end consumers and most of them are farmers who have less than 10 sapodilla trees. The rest sell their sapodilla to pickers who will sell it to big wholesalers, small wholesalers and retailers.

Table 1. Population of fruits, vegetables and medicinal in sub district of South Batipuh, 2015

| No | Commodity | Nagari (batang) | | | | Jumlah |
|---|---|---|---|---|---|---|
| | | Batu Taba | Sumpur | Padang Laweh | Malalo | |
| 1 | Sawo | 4,624 | 13,863 | 2,317 | 4,663 | 25,467 |
| 2 | Rambutan | 304 | 241 | 653 | 469 | 1,667 |
| 3 | Durian | 508 | 762 | 1,271 | 2,820 | 5,361 |
| 4 | Alpokat | 3,534 | 854 | 2,967 | 1,699 | 9,054 |
| 5 | Jeruk Nipis | 1,196 | 395 | 406 | 1,991 | 3,988 |
| 6 | Pisang | 2,878 | 1,126 | 1,102 | 3,995 | 9,101 |
| 7 | Mangga | 151 | 320 | 467 | 619 | 1,557 |
| 8 | Pepaya | 3,928 | 308 | 566 | 620 | 5,422 |
| 9 | Manggis | (123) | 7 | 45 | 1,113 | 1,042 |
| 10 | Jambu Sir | 123 | 140 | 106 | 158 | 527 |
| 11 | Jambu Biji | 29 | 54 | 45 | 168 | 296 |
| 12 | Sirsak | 1,410 | 10 | 25 | 15 | 1,460 |

Source: SP Report of Kec. Batipuh Selatan, 2015

Additionally there are many pickers in this area and most of them are the residents that don't have their own sapodilla or the pickers from outside Nagari Sumpur. The farmers tend to sell their sapodilla to pickers who buy at highest price. Thus at this level the market structure of Sapodilla is perfect competition.

At the next level, most of pickers sell sapodilla to big wholesalers (80 %) and the rest sell it to retailers and small wholesalers. This is because big wholesalers will buy at any number of productions and can provide transportation for large quantities. There are five big wholesalers of sapodilla in this area. However, small wholesalers and retailer can only purchase a limited number of sapodilla, due to the limited capital, tools and market. Although the price at small wholesaler is higher than big wholesalers, they can't buy sapodilla from farmers in large quantities. Thus at this level the market structure of sapodilla is Oligopoly competition.

3.3. The Market Conduct of Sapodilla

a. Pricing System

Since approximately 80 percent of Sapodilla from Nagari Sumpur sells to outside region of Sumatra, the price will be influenced by sapodilla production of other regions such as Tasikmalaya and Lampung. Big wholesaler in South Batipuh selatan will make a deal with wholesaler in Java. By considering the availability of supply all over the regions, cost and depreciation of the product, wholesaler in java will set the price. Thus technically farmers, pickers and big wholesalers are price takers. Consequently the price in the level of farmers and picker are also influenced by the further level of marketing.

b. Payment System

Mostly the payment system in sapodilla marketing in Nagari Sumpur is cash. The transaction between farmers and pickers is cash, between the pickers and big, small wholesalers and retailers is also cash. However the payment system between big wholesalers and wholesaler in Java is carried out through bank transfer.

3.4 The Market Performance of Sapodilla

a. Marketing Margin

The Pattern of marketing channel of Sapodilla in Nagari sumpur can be seen in Figure 1. The pattern of Sapodilla marketing channel can be categorized into the following patterns:

a. Marketing Margin of Pattern I
   Farmers → End consumers
b. Marketing Margin of Pattern II

Farmers → Big wholesalers → Wholesalers in Java → Retailers → End consumers

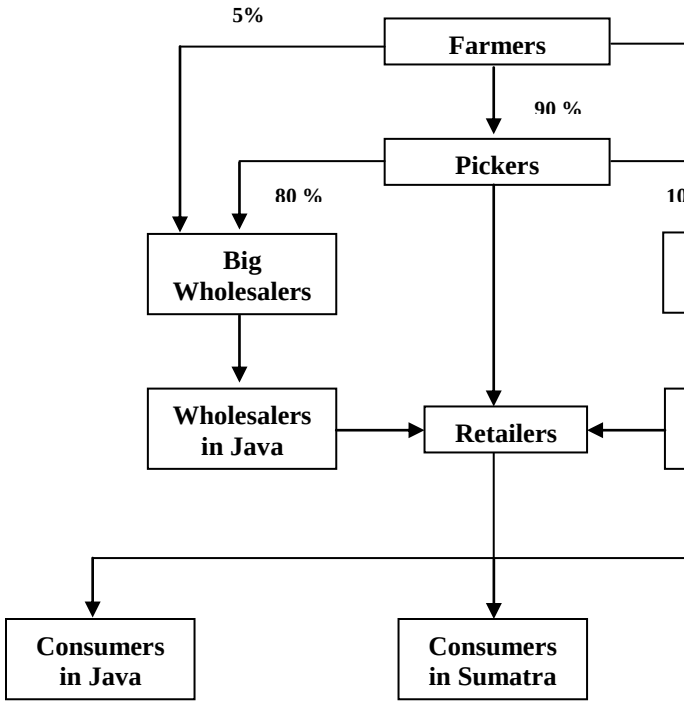


Figure 1. Marketing channel of Sapodilla in Nagari Sumpur

c. Marketing Margin of Pattern III
   Farmers → Small wholesaler → Wholesaler in Sumatra → Retailers → End consumers
d. Marketing Margin of Pattern IV
   Farmers → Pickers → Small wholesaler → Wholesaler in Sumatra → Retailers → End consumers
e. Marketing Margin of Pattern V
   Farmers → Pickers → Big wholesalers → Wholesalers in Java → Retailers → End consumers

**b. Farmer's Share**

Analysis of Farmer's share is useful to show the share received by farmers from the price paid by consumers. The farmer's share from Sapodilla marketing can be seen in Table 3. From the table above can be seen that the largest share received by farmers is in the pattern III which is 71%.

**c. Marketing Efficiency**

Marketing efficiency is the ratio of output and input values. The output value is the value of the price paid by consumers, and the input value is the cost of marketing. The most efficient marketing channel is the channel that has the smallest value of marketing efficiency.

The value of marketing efficiency on all four channels is less than 33%. This indicates that sapodilla marketin in Nagari sumpur is

Table 2. Marketing Margin of Sapodilla

| No | Pattern | Margin (%) |
|---|---|---|
| 1 | Pattern I | 0 |
| 2 | Pattern II | 47 |
| 3 | Pattern III | 29 |
| 4 | Pattern IV | 78.6 |
| 5 | Pattern V | 79 |

Table 3. Farmer's share

| Pattern | Farmer's price (Rp) | Consumer's price (Rp) | Farmer's share (%) |
|---|---|---|---|
| Pattern II | 3700 | 7000 | 53 |
| Pattern III | 5000 | 7000 | 71 |
| Pattern IV | 1500 | 7000 | 21 |
| Pattern V | 1500 | 7000 | 21 |

Table 4. Marketing efficiency of Sapodilla

| Pattern | Input value (Rp/kg) | Output value (Rp/kg) | Marketing Efficiency (%) |
|---|---|---|---|
| Pattern II | 1,531 | 7,000 | 22 |
| Pattern III | 660 | 7,000 | 9 |
| Pattern IV | 1,163 | 7,000 | 17 |
| Pattern V | 2,034 | 7,000 | 29 |

efficient. However, the most efficient one is marketing channel in pattern III which is Farmers → Small wholesaler → Wholesaler in Sumatra → Retailers → End consumers. Pattern III also provides the highest share for sapodilla farmers.

### d. Discussion

The SCP analysis shows that the marketing of Sapodilla in Nagari Sumpur is efficient. However the marketing will be more efficient and the farmers can receive the bigger market share when they sell sapodilla directly to small wholesalers. This is because the price at small wholesalers is higher than big wholesalers. However this could be difficult to be practiced, because small wholesalers can't purchase sapodilla in large quantities. Although wholesalers buy with the higher price, they have limitations in capital, tools and market.

The best alternative can be applied by farmers is selling directly to big wholesalers (pattern II). Even though the pattern IV of marketing channel (Farmers → Pickers → Small wholesaler → Wholesaler in Sumatra → Retailers → End consumers) is more efficient than pattern II (Farmers → Big wholesalers → Wholesalers in Java → Retailers → End consumers), the farmer's share of pattern II is much higher than pattern IV. In order to obtain more from sapodilla, the farmers need to pay more attention to marketing as well as the managing of sapodilla farming. Recently it is only 10 percent of farmers sell sapodilla directly to big wholesalers. So far most farmers just let their sapodilla plant with a minimum maintenance and lack of effort in marketing. This is because psychologically they realize that they did not plant sapodilla by themselves but own it by heritage. The farmers should change this mindset and do more in maintaining and marketing their sapodilla. Moreover the farmers also should do re-planting because the age of their sapodilla has already more than 75 years. Additionally, the farmers can increase income from sapodilla by its creating value added.

## Conclusion

Sapodilla is important for residents of Nagari Sumpur. There are five patterns of sapodilla marketing channels and all channels are efficient. However the most efficient channel is pattern III (Farmers → Small wholesaler → Wholesaler in Sumatra → Retailers → End consumers). However, this will be difficult to be practiced, because the limitations of small wholesalers in terms of capital, tools and market. The best alternative can be applied by farmers to gain the higher income is selling directly to big wholesalers (pattern II). Thus the farmers should pay more attention to the marketing as well as the managing of sapodilla farming.

## References

Badan Litbang Pertanian. 2012. Limapuluh Teknologi Unggulan Badan Penelitian dan Pengembangan Pertanian. Badan Peneltian dan Pengembangan Pertanian, Departemen Pertanian.

DIPTI. 1997. Analisis komoditi unggulan Propinsi Sumatera Barat. Dewan Ilmu Pengetahuan, Teknologi dan Industri (DIPTI) Sumatera Barat. Padang.

Prihatman, K. 2000. System Informasi Manajemen Pembangunan di Pedesaan. Jakarta. BAPPENAS.

Raesi, Syahyana. 2013. Strategi Pengembangan Komoditi Sawo (*Achros Zapota*, L) Di Kenagarian Sumpur Kab. Tanah Datar Sumatera Barat. Jurnal Agribisnis Kerakyatan. Unand. Padang. 2013.Volume 3. Nomor 1.

Rismunandar. 1983. Membudidayakan Tanaman Buah-Buahan. Sinar Baru. Bandung.

# Information Systems of Eradication Pests and Diseases Crops for Agriculture Extension Instructor

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## Abstract

Agricultural extension instructor of plant pests and diseases and tackling has been done by the trainers during the many difficulties and obstacles because of the location and the diversity of the problems faced. The limitations of distance and knowledge of the extension in their duties greatly affect the productivity results. To assist the government in resolving the issue, the need for a computer program information system that can be accessed in the location extension. The program should be able to address various issues of plant pests and diseases as well as mitigation. Program in accordance with the specific needs of course to be built specifically. The research result obtained is an application program that is easy to administer instructor and has been tested in the presence of pests at random extension. The test results were obtained, that the program has been good and it can be applied but need continued improvement to the development of pest control and plant diseases.

Keywords: pests, diseases, plants, agriculutural extension

## 1. Introduction

Implementation of guidance and counseling to information about plant pests and diseases as well as to overcome is the condition of the area groups of farmers. In addition, the number of existing agricultural extension workers, especially in the province of Bengkulu pretty much extension fixed, contractual or non-educator but still require additional knowledge about the prevention of pests and plant diseases. The specific objective of this study was the establishment of software (Software) which has a function as teaching material for extension workers who will go into the field in the process of guidance and counseling prevention of pests and plant diseases. So knowledge of the extension can always be renewable, in accordance with the development of information about the prevention of pests and plant diseases.

The resulting software will be delivered master freely to the government through a coordinating body extension (Bakorluh) Bengkulu Province as a pilot project of the program management information systems to control pests and plant diseases. While the use of the training program will be held following the schedule set by Bakorlah after receiving instructions from the provincial government. The general objective of this study was to achieve an increase in productivity of agriculture in each area farming groups get assistance from counselors who have been equipped with the knowledge of prevention of various pests and plant diseases. In other words, its relevance to the increase in household income after the farmers free of pests and plant diseases. So that the agricultural extension instructor can answer the various issues raised by farmers in terms of the symptoms caused by pests and diseases

that attack the crop and countermeasures in accordance with the recommendations of experts through this program.

Based on the above facts, then that need attention later on is the level of completeness of the data and information relating to the issue of pests, diseases that attack the crop and at the same time various procedures for handling. Thus updating the data and the information was continuously indispensable for renewed (updatable) and delivered to farmers belonging to the groups of farmers.

## 2. Material and Methods

Information systems

Information is a collection of data that is processed into a form that is more useful and more meaningful for those who receive. Without the information, the system will not run smoothly and will eventually die, in other words, is the data source for the information. The information system is a system within an organization reconcile the needs of the processing of daily transactions that support the function of the organization's operations that are managerial in strategic activities of an organization to be able to provide to outside parties certain information necessary for decision-making and may also be information for all levels in such organizations whenever necessary. An information system is a collection of hardware and computer software and hardware man will cultivate and use. In addition the system can be defined following information:

1. A system created by humans which consists of components within the organization to achieve a goal that is present infornasi.
2. A set of organizational procedures when implemented will provide information for decision makers and or to control the organization.
3. Reconciling the needs of transaction processing, support the operation, managerial and strategic activities of an organization and provide certain outside parties with the necessary reports.

Plant Pests and Diseases

The pest is a plant cultivated vermin eg rice, wheat, potatoes, mangoes, apples and so on. While the disease is causing the plant to be sick, such as bacteria, fungi, viruses, lack or excess of water. While the pain is a condition deviating from normal. Having knowledge of pests and diseases has been held, further control of pests and diseases will provide very good impact on crops and productivity. While the action taken is in the form of biological by providing pest predators. (Pracaya, 2007).

Model designing a system reveals that there are several models that can be used, namely :

1). Waterfall Model (Waterfall), which is a model that describes the system design complete stages ranging from analysis and requirements definition, system design and software, implementation and unit testing, integration and system testing, operation and maintenance.
2). Evolutionary Development Model, which is a model which is based on the idea and initial diimplemetasi then offered to customers to be explored and commented upon. Then gradually revised in accordance with the wishes of the user.
3). Literature study, which searches a variety of information on plant pests and diseases that have been published by the experts and latest.

## 3. Result and Discussion

The results obtained in this study was the establishment of an information system program pest control and plant diseases. The display of the program after the run looks as follows :

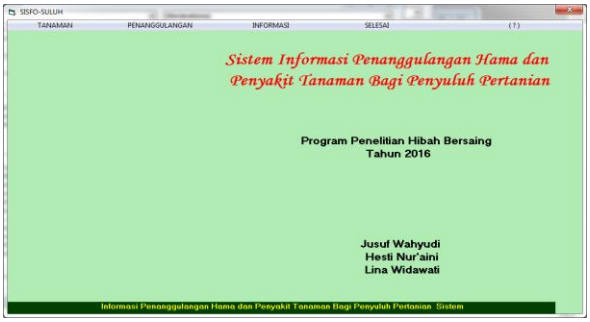


Figure 1. Main Program

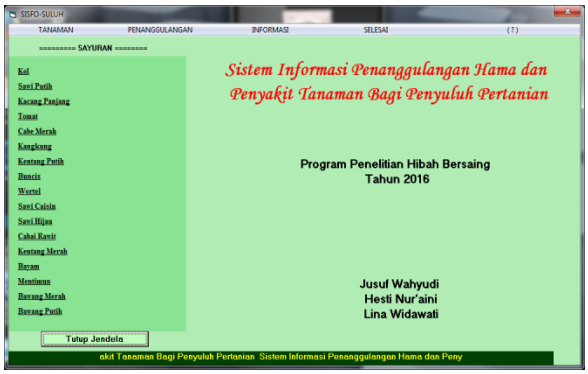


Figure 2. Submenu Crops - Horticulture - Vegetables

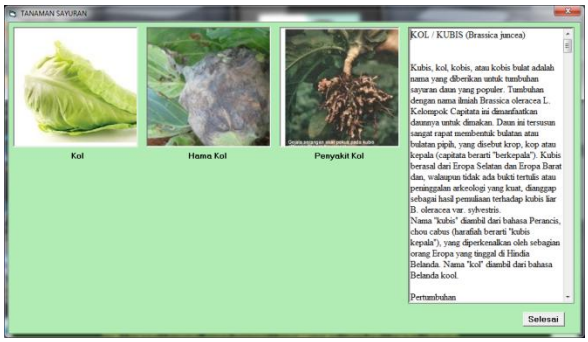


Figure 3. Example of Vegetable Cabbage with Pests, Diseases and short explanation

Figure 1 above is a result of a program that has been created, in which the program is organized into the menu, namely: Plants, Prevention, Information and Done. Tanaman (Plants) submenus are provided to serve the various problems of the existing plant, submenus Penanggulangan (Eradication) provided to serve the needs of penanggulapungan both pests and diseases of plants is desired.

Information submenu are provided to serve the needs in terms of information about the desired plants. So with these programs, agricultural extension can freely assist farmers who need a variety of information related to pests and diseases as well as ways to overcome them.

Based on the display as figure 1 above, visible program has several menu options like Crops, Eradication, Information and Done as could be seen in Table 2 – Table 5.

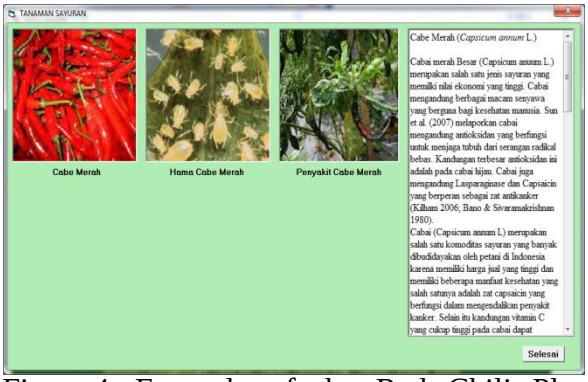


Figure 4. Example of the Red Chili Plant Pests, Diseases and short explanation

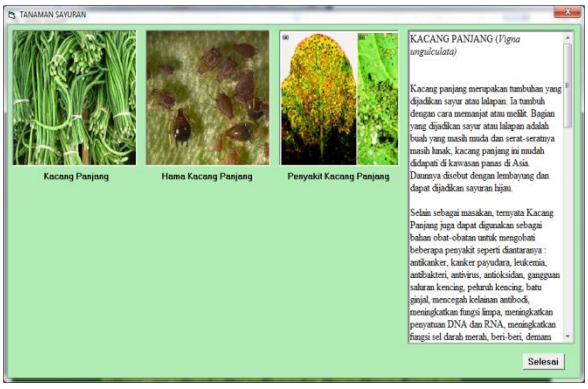


Figure 5. Example Vegetable Long Bean with Pests, Diseases and short explanation

The following figures are shown some examples for fruit crops:

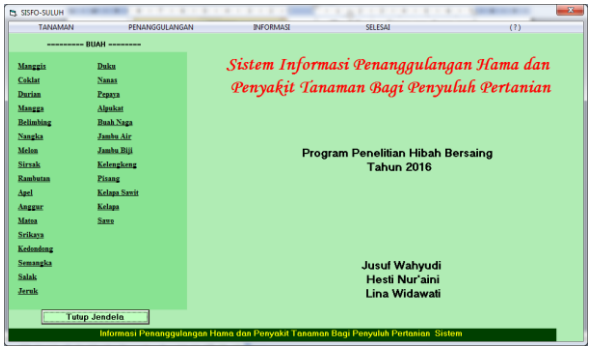


Figure 6. Example Display sub-menu At Fruit Program

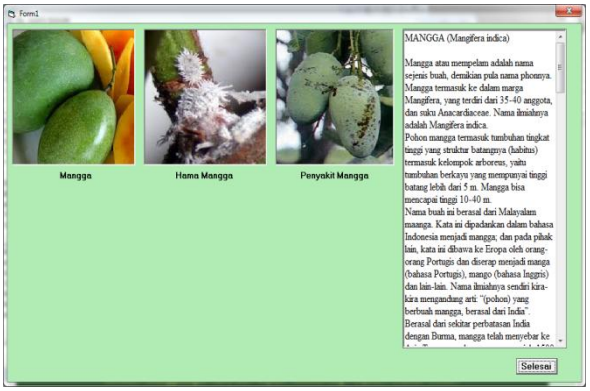


Figure 7. Example Fruit Mango with Pests, Diseases and short explanation

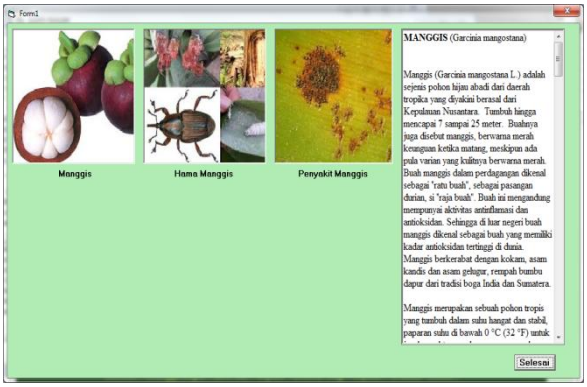


Figure 8. Example Fruit Mangosteen with Pests, Diseases and short explanation

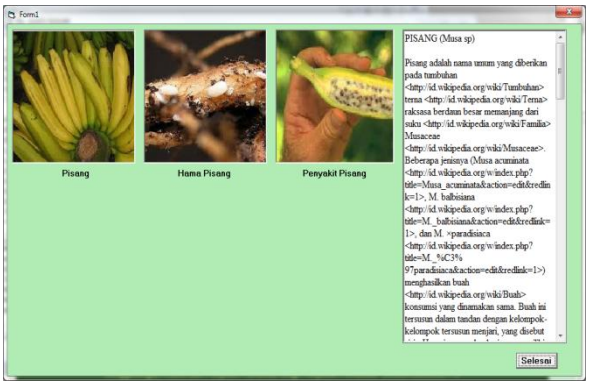


Figure 9. Example Fruit Banana with Pests, Diseases and short explanation

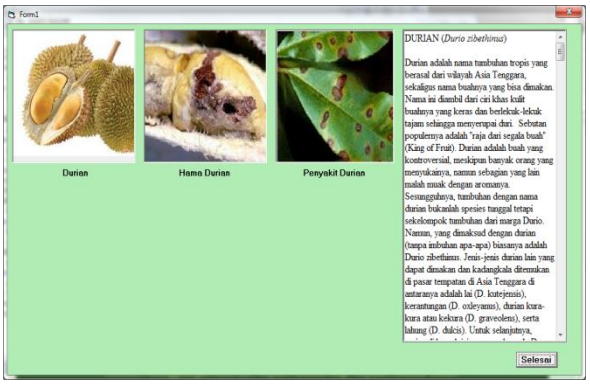


Figure 10. Example Fruit Durian with Pests, Diseases and short explanation

## Conclusion

Based on the results of the testing program that has been created, the comments provided by the extension of plant pests and diseases can be described as follows:

1). Need additional information related to pests and diseases and tackling the various other plants.
2). So that the program can be optimized, it is necessary the addition of some facilities, such as printing brochures concise, facility updates and discussion forums

## References

Kristanto, A. 2008. Design of information systems and applications. Gava Media. Yogyakarta.

Anonymous. 2013. Agricultural statistics human resources and institutional data center 2013. Farmers and Agricultural Information System.

Secretariat General of the Ministry of Agriculture ……..

Novia, A. 2007. The windows applications with visual basic enterprise. PT. Elekmedia Komputindo. Jakarta.

Nathasia, N. D. 2013. Design expert system identification of horticultural crops disease prevention to facilitate hama. Journal of Information Technology. Volume 2. No. 2 of 2013. http: // lkppm. pradnya.ac.id/wp-content/uploads/2013/03/6.-pakar-tanaman-168-180.pdf. Downloaded on April 24, 2014.

Pracaya, 2007. Plant pests and diseases. Revised Edition. Penebar Organization. Jakarta. 434 Pages.

Rusmawan, UUS. 2009. Collection program concept ADO VB 6.0. PT. Elex Media Komputindo. Jakarta.

Sutanta, E. 2005. Introduction to information technology. Graha Science. Jakarta.

Siswanto, Abdul Fadlil, 2013. The expert system for diagnosing plant pests and diseases onion using certainty factor. Journal of Bachelor of Engineering Informatics, 1, 2338-5197.

Winardi, 2010. Principles of management principles. CV. Mandar Maju. Bandung.

# The Regional Investment Competitiveness In Binjai City

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## Abstract

Regional Investment Competitiveness became one of the issues in regional development policy since the enactment of local autonomy. Good investment climate will encourage economic growth. The investment climate in Indonesia is still not good. Rank position of Indonesian investment competitiveness is still at the lower ranked among other countries. This study aims to identify the level of investment competitiveness in Binjai City. Measuring the level of competitiveness of the investment is made by using primary data were analyzed through qualitative scoring rankings. The indicators used in this study include the regional economy, human resources, local infrastructure, institutional and government as well as financial institutions. The results show the institutional and governance indicators are indicators which have the lowest levels of competitiveness among the five other indicators. Aspects of government support on tax incentives, licensing fees and corruption are aspects that inhibit investment in improving the competitiveness of Binjai City.

Keywords : Investment, Regional Competitiveness

## 1. Introduction

Regional development is a part of national development. Regional development is a planned effort to improve local government capacity so as to create an ability to provide public services, and the ability to manage resources efficiently regional economic and effective for the region's economic progress and prosperity.

Since regional autonomy began in 2001 ago, aspects of development and regional planning more necessary and decisive in the national development process. This is because the authority of local governments to manage development in their respective regions becomes larger.

In the era of regional autonomy, the district / city in Indonesia addressing the issue of economic development in the region. In addressing the issue of economic development of the region must build economic competitiveness and efficient. In the era of regional autonomy, the economic development program should be decentralized and competitive, so the scope is broader and not just local economic development [1].

In the area of economic development, a target of increasing economic growth into one of the very important purpose. One factor supporting economic growth is investment. Investment is one of the important factors determining the success of economic development. Investment is one of the main macroeconomic indicators that can be used to measure the performance of a regional economy. The existence of investment is the basis for the realization of sustained economic growth.

How big investments gained strongly related to how local governments increase the attractiveness of investors to invest in the area. The appeal is called the investment

climate. Favorable investment climate will encourage economic growth, through investment supported by high productivity. The investment will strengthen economic growth by bringing more inputs into the production process. Therefore, improving the investment climate is an important task for every government, especially countries that have low investment competitiveness such as Indonesia.

In recent years, the investment climate in Indonesia is still cause for concern. Some of the results of a survey of international organizations, shows that the ranking position of Indonesian investment competitiveness is still at the lower ranked group. This shows the seriousness of the problem of investment climate that must be addressed. Therefore, improving the investment climate is an important task for any local government.

Lack of competitiveness due to the low quality of service bureaucracy, inefficient businesses, rising labor costs, the poor quality of infrastructure and the high cost of investment in Indonesia [2]. The level of competitiveness is one of the parameters in the concept of sustainable regional development. The higher the level of competitiveness of a region, the increase in welfare even higher.States that the investment will go into an area will depend on the competitiveness of the investment held by a related region [3].

Binjai is one of the city directly adjacent to the provincial capital of North Sumatra. Binjai is one of the main areas for economic growth hitterland North Sumatra in general and a place to invest is still potential.

With the implementation of regional autonomy expected Binjai city became the center of a new force in the field of economic, social and political. Binjai is one of the cities that are accelerating development in all fields, which aims to attract investors both domestic and foreign investors. Binjai potential and opportunities in attracting investors is quite large. Almost all sectors of the economy in Kota Binjai has the potential to be developed. Prospects for investment in Binjai is also promising, because the location of the strategic city of Binjai, near the provincial capital of North Sumatra.

This study aims to determine the extent of the investment climate conditions Binjai so it will be able to have better competitiveness in the future.

## 2. Material and Methods

Data analysis was performed using descriptive statistics. The data used is the qualitative primary data which was later changed to quantitatively using scoring and ranking measurement scale. The analysis technique of scoring the scoring against the respondents to obtain quantitative data required.

Investment competitiveness is analyzed using a number of indicators that reflect the determinants of investment. The indicators used in the assessment of the competitiveness of investments in Kota Binjai include 1) Regional Economy, 2) Human Resources, 3) Regional infrastructure, 4) Institutional and Government, as well as 5) Financial Institutions.

Rate indicator of level of competitiveness of the investment is done by providing an assessment of the various indicators with a scale of 1-7. Rating 1-7 means the assessment of indicators ranging from severely hampered by the very supportive.

## 3. Result andDiscussion

Based on data collection in the field consisting of government agencies, academia, business associations, as well as the businesses themselves obtained that level of investment competitiveness in Kota Binjai on the value of 4.27 of a scale of 7. This value indicates that investment activity in Binjai competitiveness is not optimal.

Table 1. Indicator of Regional Investment Competitiveness

| No | Indicator | Value |
|---|---|---|
| 1 | Regional Economy | 4,02 |
| 2 | Human Resources | 4,31 |
| 3 | Regional infrastructure | 5,05 |
| 4 | Institutional and Government | 2,79 |
| 5 | Financial Institutions | 5,2 |
| | Average | 4,27 |

Source : Primary data (processed)

Table 2. Indicators of Regional Economy

| No | Indicators of Regional Economy | Value |
|---|---|---|
| 1 | Regional Economic Climate | 4,40 |
| 2 | Output price stability | 3,77 |
| 3 | Input Price Stability | 3,90 |

Source : Primary data (processed)

Table 3. Indicators of Human Resources

| No | Indicator of Human Resources | Value |
|---|---|---|
| 1 | The availability of local Labor competent | 4,63 |
| 2 | Ethos of Labor | 4,43 |
| 3 | Labor Productivity | 4,33 |
| 4 | Quality of Local Labor | 4,40 |
| 5 | Minimum wage levels Regional | 3,73 |

Source : Primary data (processed)

This condition indicates that the need for a policy and program to be able to improve the competitiveness of investments in Kota Binjai so that later can become the driving force of improvement of regional competitiveness regionally and internationally.

*3.1. Indicators of Regional Economy*

Regional economic factor is an indication of the economic potential and economic structure of a region which is an important consideration in supporting the competitiveness of local investment. Regional economic factors will affect the development of investment activity in the area. Assessment of regional economic indicators covering several aspects of the economic climate of the region, the stability of output prices and input price stability. According to respondents, the economic climate of the area Binjai have good conditions compared to the situation of price stability input and output prices.

*3.2. Indicators of Human Resources*

Human resources as one of the factors of production other than natural resources, capital, entrepreneurs need to generate output. The higher the quality of human resources, it also increase the efficiency and productivity of an area. The number and quality of its human resources of a region able to provide sufficient impetus decisive significance in enhancing investment in an area. The quantity and quality of the results of the investment activities is determined how well the condition of human resources has a role as actors in economic activities so that eventually able to create an increase in output in an area.

According to the survey, found that the availability of local workforce competent in Binjai is enough available as required in investment activities, despite the availability of a competent workforce does not exceed the needs of the business world. Once the work ethic, productivity and quality of labor in Binjai is sufficient to support investment activity, although in value that must be improved in order to be able to satisfy the needs of investment activities and competence with workers from outside the city.

*3.3. Indicator of Regional infrastructure*

Infrastructure is one of the key determinants of the regional economy. The availability and quality of infrastructure is a very important factor remedy attracting investment. Promotion strategy to attract

investment interest would be meaningless without the provision of adequate infrastructure. Provision of infrastructure into consideration tersediri to be considered in investment due to the availability of infrastructure is able to expedite market access, communication and business activity the better.

### *3.4. Indicators of Institutional and Government*

Region's ability to attract investors in investing in an area can be seen from the regulations created by local governments. The longer and more expensive regulatory processes created by an area will have an impact on the investment slowdown or even a decline in investment in the region. This condition means that the area has no appeal for investors who ultimately will have an impact on slowing output and economic growth in the region.

In general, institutional and governmental factors still an obstacle or a barrier to improving the competitiveness of investments in Binjai. According to respondents, the institutional and governance factors that are currently happening in the city of Binjai made investors quite expensive in investment activity. The licensing process is relatively long, which is pretty much requirements licensing, licensing costs beyond what is estimated as well as corruption could inhibit investment activity in Binjai. Thus the need for the improvement and enhancement of the whole institutional and governance factors in Binjai.This is consistent with survey results ASEAN-BAC (ASEAN-Business Advisory Council) from 2011 to 2012 in the 405 businessmen mentioned that the main factor which becomes a barrier investment in Indonesia is corruption [4].

### *3.5. Indicators of Financial Institutions*

The availability and quality of financial institutions in the region will also be able to influence the investment activity. This is because financial institutions become major support institutions for investment in the process of payment of financial transactions conducted by the company both with consumers in the city and outside the city. In addition, the financial institution is able to make an impact for the development of investment through crediting for business development at businesses to business activities that increasingly growing.

Binjai already have a good financial institution with a good quality good. Availability of financial institutions in Kota Binjai not only visible from the many existing financial institutions but also of a variety of banking products which are capable of supporting the investment climate in Binjai. In detail can be seen in the table below.

Table 4. Indicators of Regional Infrastructure

| No | Indicators of Regional Infrastructure | Value |
|---|---|---|
| 1 | Quality of Road | 4,80 |
| 2 | Availability of Electricity | 4,30 |
| 3 | Quality Access Communications | 5,30 |
| 4 | Availability of Water | 4,83 |
| 5 | Quality of Port | 5,07 |
| 6 | Quality of Airport | 5,63 |
| 7 | Internet Network Quality | 5,40 |

Source : Primary data (processed)

Table 5. Indicators of Institutional and Government

| No | Indicators of Institutional and Government | Value |
|---|---|---|
| 1 | Old Time Management Licenses | 3,13 |
| 2 | Licensing Requirements | 3,17 |
| 3 | Licensing fees | 2,80 |
| 4 | Corruption | 2,90 |
| 5 | Government Support in Tax Incentives | 1,97 |

Source : Primary data (processed)

Table 6. Indicators of Financial Institutions

| No | Indicators of Financial Institutions | Value |
|---|---|---|
| 1 | availability of financial institutions | 5,27 |
| 2 | Variations Banking Products | 5,07 |
| 3 | Product Variations Other Financial Institutions | 4,60 |
| 4 | Distribution Branch Office | 5,13 |
| 5 | Quality of Banking Services | 5,63 |
| 6 | Ease of e-Banking Transaction | 5,77 |
| 7 | Quality of Banking Information System | 5,53 |
| 8 | Mortgage interest against income | 4,37 |

Source : Primary data (processed)

## Conclusion

Institutional and governance indicators are indicators which have the lowest levels of competitiveness among the five other indicators. Aspects of government support on tax incentives, licensing fees and corruption are aspects that inhibit investment in improving the competitiveness of Binjai.

## References

[1] Subandi, *Economic Development*, Ist Edition, Alfabeta, Bandung, 2011.
[2] Kuncoro, M., Autonomy and Regional Development. Erlangga, Jakarta, 2004.
[3] KPPOD, *Investment Attractiveness 214 regencies / cities in Indonesia in 2005*. Jakarta, 2005.
[4] Indriani, Agni, *Investment in Indonesia(*2016) homepage on depkeu [online]. Available : http://http://www.bppk.depkeu.go.id/webpkn/attachments/791_5-Investasi di Indonesia-Agni-Edited by Tanda-EDIT OK.pdf.
[5] Jhingan, M.L. *Economic Development and Planning*, 1st . Rajawali Pers. Jakarta.2007.
[6] Mankiw, N. Gregory. *Macroeconomic Theory*, 6th edition. Erlangga. Jakarta. 2006.

# The Impact of Rising Soybean Prices to Tofu Industry Small Scale in Medan

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## Abstract

Tofu industry in Medan has small-scale enterprises with limited budget so that when the price increases it will affect business conditions of tofu. This study aims to determine the impact of rising soybean prices to tofu industry small scale in Medan. This research use the case study method (case study). The analytical method used is descriptive method.The increase in soybean prices by 13% (2010-2014) led to price of tofu an increase of 15.97% and has an impact on tofu volume production decreased by 17.44%. The decreased in production volume led to receipts decreased by 4.26%. tofu industry revenue amounted to 40.12% or from Rp.2.015.199 be Rp.1.206.765. The tofu maker anticipate the increase of soybean prices by increasing selling prices to cover production costs and reduce the size of tofu in order to keep the customers.The rated of R/C on total costs decreased by 12.54% from 1.38 in the condition before the increase in soybean prices to 1.21 on the condition after the increase in soybean prices. Tofu enterprises are still possible to be run because the value of R/C is more than one, but the decreased in the value of R/C indicates that the income of tofu maker will decrease.

Keywords: soybean price, tofu industry, income analysis, R/C ratio.

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## 1. Introduction

Agricultural commodities in general are easily damaged so it needs to be consumed directly or processed first. Called agro-industrial processing, can increase the added value of agricultural commodities. Agro-industrial activities is an integral part of the development of the agricultural sector. the agro-industry is able to transform primary products into refined products. Primary products that do not have added value, into products that have high added value.[1]

Soybean (Glycine max) is a commodity that has long been cultivated in Indonesia and the prospect of its development is still very bright. This gives a hint that soy has an economic and social value is high, and the increasingly strategic role in the order of human life. Soybean is an agricultural commodity that is urgently needed in Indonesia, both as an ingredient in human food, animal feed, industrial raw materials, as well as refresher material. Soybeans contain high levels of 40% protein and 10-15% fat. Until now soy is still a vegetable source of food protein most cheap so it is not surprising that soybean demand total for the food reaches 95% of the total demand of soybean in Indonesia.[2]

Table 1. Soybean Production Development of North Sumatra Province Years 2010-2014

| Years | Harvested area (Ha) | Production (Ton) | Productivity (Kw/Ha) |
|---|---|---|---|
| 2010 | 7.803 | 9.439 | 12,10 |
| 2011 | 11.413 | 11.426 | 10,01 |
| 2012 | 5.475 | 5.419 | 9,90 |
| 2013 | 3.126 | 3.229 | 10,33 |
| 2014 | 5.024 | 5.705 | 11,36 |

Source: The Ministry of Agriculture is processed in 2015

Table 2. Domestic Developments in the Production, Import and Supply 2010-2014 National Soybean

| Years | Domestic Production | | Import | | Supply | |
|---|---|---|---|---|---|---|
| | Ton | % | Ton | % | Ton | % |
| 2010 | 907.000 | 34,26 | 1.740.505 | 65,74 | 2.647.505 | 100,00 |
| 2011 | 851.000 | 28,95 | 2.088.616 | 71,05 | 2.939.616 | 100,00 |
| 2012 | 843.000 | 12,75 | 5.767.015 | 87,25 | 6.610.015 | 100,00 |
| 2013 | 780.000 | 12,74 | 5.341.159 | 87,26 | 6.121.159 | 100,00 |
| 2014 | 921.000 | 21,47 | 3.367.977 | 78,53 | 4.288.977 | 100,00 |

Source: Secretariat General Agricultural processed in 2015

In 2010, soybean production in North Sumatra Province reached 9.439 tonnes to 5.705 ton in 2014. The decline in harvested area in line with productivity and soybean crop production in Indonesia on the wane. This is due to the conversion rate of agricultural land in North Sumatra, which continues to increase, especially in the central area of soybean production. Conversion of land which led to reduced production of soybean in North Sumatra is certainly more difficult for the government to meet the demand of soybean that can not be covered by the existing supply that needs to increase soybean imports.

Soybean production continues to decline each year causes the level of dependence on soybean imports, Indonesia is likely to increase from year to year. 2014 Indonesian soybean imports reached 78.53 percent or 4,288,977 tons, while production in the country reached 21.47 percent, or 921,000 tons. soybean demand is high but can not be met by the availability of local soy as an alternative cause of soybean import volume is increasing every year. Indonesian government's dependence on imported soybean increased, causing soybean as raw material for making business out also increased.

The study results showed that tofu of protein-rich high-grade, high-protein complementation properties, ideal for food diet, low in saturated fat and cholesterol free, rich in minerals and vitamins, natural foods are healthy and free of chemical compounds poisonous.Tofu is easily damaged or rotten.[3]

Soybean prices affect the manufacturers tofu, soybean price increase occurred due to import soybeans from other countries which are the raw material of tofu as one of the specialties in Indonesia. Rising soybean import tariffs pretty bad impact on the Indonesian economy rather than the terms of trade. Rising soybean prices caused by several factors, among others, domestic production is still minimal, the rupiah against the US dollar and the effects of erratic weather.[4]

Table 3. Local Soybean Prices in North Sumatra 2010-2014

| Month | Unit | Years | | | | |
|---|---|---|---|---|---|---|
| | | 2010 | 2011 | 2012 | 2013 | 2014 |
| Jan | Rp/Kg | 7.000 | 8.033 | 7.875 | 9.260 | 8.100 |
| Feb | | 6.833 | 8.367 | 7.875 | 9.060 | 8.267 |
| Mar | | 7.000 | 8.367 | 7.875 | 9.000 | 8.267 |
| Apr | | 7.000 | 8.367 | 7.750 | 9.000 | 8.267 |
| Mei | | 7.167 | 8.000 | 7.750 | 8.900 | 8.267 |
| Jun | | 6.850 | 8.000 | 7.875 | 9.350 | 8.100 |
| Jul | | 7.017 | 8.000 | 7.800 | 9.110 | 8.267 |
| Agu | | 7.183 | 8.000 | 8.063 | 9.900 | 8.267 |
| Sep | | 7.183 | 8.167 | 8.500 | 11.100 | 8.433 |
| Okt | | 7.017 | 8.167 | 8.500 | 10.460 | 8.350 |
| Nop | | 7.350 | 8.500 | 9.250 | 10.460 | 8.600 |
| Des | | 7.417 | 8.167 | 9.300 | 10.460 | 8.767 |
| Average | | 7.085 | 8.178 | 8.201 | 9.672 | 8.329 |

Source: BPS, Statistics Producer Price Processed in 2016

Based on the development of local soybean prices in North Sumatra from the year 2010 - 2013 is constantly increasing prices ranging from Rp. 7,085 / kg to Rp. 9672 / kg. Then declined in the following year is Rp. 8329 / kg. It is very impacting to smooth the business for manufacturers of tofu because this industry has a small scale with a small capital and access to loan funds are also limited. Medan city has several industrial centers tofu spread over several districts. One area is the famous district of Medan Deli and several other areas such as District of Medan Marelan, District of West Medan, District of Medan Petisah, District of Medan Polonia, and the District of Medan Selayang.

## 2.Research Methods

This method uses the case study method (case study) research that is used to look directly into the field, as the case study is a method that describes the type of research on a specific object during the period.

### *2.1 Methods Siting*

Determination of the study area is purposive sampling .or intentionally, namely in the city of Medan. Medan is not a production center soybeans, but in Medan there are many industrial businesses tofu that already run successfully for many years.

### *2.2.Sampling Methods*

The sample in this study is the industrial business owners tofu that produce white tofu small scale in the city of Medan.and is willing to be interviewed. Selection of the samples used in the industry tofu this study using nonprobability sampling technique, namely snowball sampling.

### *2.3. Analysis Method*

The analytical method used in this research is descriptive method.. The analysis was conducted in the form of profit analysis, receipt and analysis of R / C ratio. To determine the amount of profit-making business idea can be calculated using the formula (Kasim, 2004). Calculation analysis of the following advantages:

$I = TR - TC$

TC = FC + VC

I = Income
TC = Total Cost
TR = Total Revenue
FC = Fixed Cost
TC = Total Cost
VC = Variable Cost

Analysis of R / C ratio or the balance of receipts and expense analysis is a comparison between the number of admissions to the total outlay. The larger the value of R / C, the better the business. To generate success rate craftsmen, Mathematically it can be used the following formula:

$$RCR = \frac{Total\ Revenue}{Total\ Cost}$$

the decision-making criteria as follows:

a. RCR> 1, the effort is worth it.

b. RCR = 1, the business is not profitable but did not suffer losses.

c. RCR <1, the effort is not worth it.

## 3.Results and Discussion

Analysis of operating income is the difference between revenue and business expenses that occurred during the study. Then compared with a profit after the price increase soybean 2012-2013. How to calculate the costs and revenues are nominal approach does not take into account the time value of money. In this case the change is the price of soybeans, while other prices are considered fixed.

The increase in soybean prices affect business conditions, especially the industry of tofu in Medan, both directly and indirectly. These impacts include the use of raw materials, production volume, selling prices, business expenses, revenue and income craftsmen of tofu.

Soybean is the main raw material tofu and hold the largest percentage in the cost of production so that the increase in soybean prices led to the tofu and difficulties in running its business. Here are more details can be seen in Table 4.

According to the table 4 can be seen the condition before and after the increase in soybean prices. Prior to the increase in soybean prices, the average use of 575 kg of raw material. While the condition after the increase in soybean prices average use of raw materials decreased by 492 kg. The increase in soybean prices in 2012-2013 led to the reduction of raw materials amounted to 14.43%. Based on the weight of tofu or soybean raw material use to output per box tofu where the condition before increasing soybean prices was 1.56 kg / box, meaning that every production tofu perbox using soybean raw material of 1.56 kg. While at the time conditions after the increase in soybean prices, weights out an increase of 3.84% ie 1.62 kg/box.

### *3.2 Impact on Production Costs*

The cost analysis is required by each business activity to determine the amount of funds that must be spent to make a product. Similarly, the industrial enterprises tofu in Medan. Soybean is the main raw material tofu and hold the largest percentage in the cost of production so that the increase in soybean prices led to the tofu impediment in running the business.

### *3.1 Impact on Use of Raw Materials*

Table 4. Percentage Use of Raw Material Preparation Tofu/ Box

| Componen | Before increase Soybean Prices | After Increase Soybean Prices | Percentage (%) |
|---|---|---|---|
| RawMaterial(kg) | 575 | 492 | -14,43 |
| Production (box) | 367 | 303 | -17,43 |
| Composition soybean(Kg/box) | 1,56 | 1,62 | 3,84 |

Source: Primary Data processed in 2016

Table 5. Use of Total Production Costs Making Tofu

| Component | Before increase Soybean Prices (Rp) | After increase Soybean Prices (Rp) | Persentage (%) |
|---|---|---|---|
| Variabel Cost (VC): | 5.137.987 | 5.636.710 | 9,71 |
| - Soybean | 3.876.667 | 4.420.833 | 14,04 |
| - Labor | 789.167 | 789.167 | - |
| - Chioko | 39.700 | 33.300 | -16,12 |
| - Solar | 86.870 | 76.160 | -12,33 |
| - Firewood | 215.000 | 190.833 | -11,24 |
| - Plastic | 30.583 | 26.417 | -13,62 |
| - Transport | 100.000 | 100.000 | - |
| Fixed Cost (FC): | 125.525 | 125.525 | - |
| - Electric | 53.333 | 53.333 | - |
| - PBB | 3.300 | 3.300 | - |
| - Building Rent | 40.000 | 40.000 | - |
| - Depresiasi | 28.892 | 28.892 | - |
| TC(VC + FC) | 5.263.512 | 5.762.235 | 9,47 |

Source: Primary Data processed in 2016

The largest costs in the business of making tofu is the cost for the purchase of raw material soybeans. Costs incurred for raw material soybeans before the increase in soybean prices is Rp. 3.876.667 and after the increase in soybean prices for the raw material costs increased by 14.04% ie Rp. 4.420.833.

According to the table 6. can be seen that the cost of raw materials of soy per box before the price increase soybean Rp. 10.753. Meanwhile, after the increase in soybean prices, raw material costs increased 37.85% in the amount of Rp.14.574. The total cost of making tofu per box increased by 30.95% ie at the time before the rise in soybean prices the total cost per box is Rp. 14.355, then after the increase in soybean prices the total cost per box is Rp. 18,997. This makes the tofu anticipate by how far tofu per box size and increase the selling price per box tofu so as to cover increased production costs.

*3.3 Impact on Production and Pricing*

The increase in soybean prices in addition to impact on the use of raw material soybeans also affects the volume of production and the selling price of tofu. For more details can be seen in Table 6.

On average tofu produced in one production process is 367 boxes at a price of Rp. 19,833 per box, while the condition after the price increase average soybean tofu production during one process is 303 boxes at a price of Rp. 23,000 per box. The increase in soybean prices led to total production of tofu decreased by 17.43% while the price of tofu per box rose only 15.97%. The tofu and soybean prices rise anticipate that by increasing selling prices tofu to cover production costs and reduce the size of tofu with the aim of maintaining customers.

Table 6. Production and Pricing Tofu Before and After Price Increase Soybean

| Component | Before increasing Soybean price | After increasing Soybean price | Percentage (%) |
|---|---|---|---|
| Production (box) | 367 | 303 | -17,43 |
| Price (Rp/box) | 19.833 | 23.000 | 15,97 |

Source: Primary data processed in 2016

Tabel 7. Industry Revenue Tofu Before and After Price Increase Soybean

| Componen | Before Increase soybean prices | After Increase soybean prices | Percentage (%) |
|---|---|---|---|
| Production(box)(Q) | 367 | 303 | -17,43 |
| Price (Rp/box) (P) | 19.833 | 23.000 | 15,97 |
| Revenue (P.Q) | 7.278.711 | 6.969.000 | -4,26 |

Sources: Primary data processed 2016

*3.4 Impact of Revenue*

Reduction in the number of production causes the reception average of tofu industry decreased. To more clearly seen in Table 7.

Based on the above table it can be seen that a decline in reception where the condition before the price increase soybean receipts of Rp. 7,278,711, while after the increase in soybean prices caused revenue to drop to 4.26% from Rp. 6.969 million

*3.5 Impact On Income*

Lower revenue caused income entrepreneurs also decreased. Income is the difference between revenue and total costs. The total income affect the amount of earned income entrepreneurs. Revenue to be one way of measuring the success of a business. A business that is run will always expect revenue or maximum profits earned. Based on the survey results in the study, tofu confessed reduced revenue after the increase in soybean prices. It is more due to a production which also reduced. Soybean prices are constantly rising costs and craftsmen higher earned income decreases. To more clearly seen in Table 8.

According to the table 8. shows that with the increase in soybean prices will affect income earned tofu in Medan. Based on the above table shows that the income received craftsmen decreased. This is because the craftsmen reduce the amount of production, but the increase in selling prices slightly. On the condition before the price increase soybean industry revenue reached Rp. 2,015,199 each one of production but after the price increase revenues soybean industry knows decreased 40.12% in the amount of Rp. 1.206.765. To see revenue / profit per box of tofu industry can be seen in Table 9.

Table 8. Income of Tofu Industry Before and After Price Increase Soybean

| Component | Before Increase soybean prices | After Increase soybean prices | Percentage (%) |
|---|---|---|---|
| Total Cost (TC) | 5.263.512 | 5.762.235 | 9,47 |
| TotalRevenue (TR) | 7.278.711 | 6.969.000 | -4,26 |
| Income (TR-TC) | 2.015.199 | 1.206.765 | -40,12 |

Source: Primary data processed in 2016

Table 9. Income of Tofu Industry Before and After Increase Soybean Price per Box

| Component | Before Increase soybean prices | After Increase soybean prices | Percentage (%) |
|---|---|---|---|
| Cost/box (Rp) | 14.355 | 18.997 | 30,95 |
| Price/box (Rp) | 19.833 | 23.000 | 15,97 |
| Income/box (Rp) | 5.478 | 4.003 | -23,31 |

Source: Primary data processed in 2016

Table 10. Analysis of R / C Ratio Industry Tofu

| Component | Before Increase soybean prices | After Increase soybean prices | Percentage (%) |
|---|---|---|---|
| Total Cost | 5.263.512 | 5.762.235 | 9,48 |
| Revenue | 7.278.711 | 6.969.000 | -4,26 |
| Analysis R/C | 1,38 | 1,21 | -12,54 |

Source: Primary data processed in 2016

According to the table 9. shows that with the increase in soybean prices will affect industry revenues tofu. Based on the above table shows that the tofu industry revenue to decline. This is because an increase in production cost per box is bigger (30.95%) compared to the sales price increase (15.97%). On condition before soybean prices increased revenue earned per box tofu Rp. 5478, however, after price increases soybean tofu industry revenue declined 23.31% in the amount of Rp. 4,003.

*3.6 Feasibility Analysis of R / C Ratio*

The feasibility analysis that is used is the analysis of admission fees or R / C ratio. Analysis of the balance between the total revenue with total cost of testing is a type of business profits. The criteria used in this analysis is that if the value of R / C is greater than one, then the business is said to be lucky and be eligible to run for the amount of revenue is greater than the costs incurred. Rated R / C is smaller than one, then the business is said to be losing money and is not eligible to run because of the amount of revenue is less than the costs incurred. R / C is equal to one then venture out to experience the break-even point or a break even venture because total income is equal to total costs. The following table analyzes the R / C ratio of the industry tofu.

Based on table 11 it can be seen that the value of R / C on total costs decreased by 12.54%. Prior to the increase in soybean prices the value of R / C on the total cost was 1.38, meaning that for Rp 1.00 cash cost incurred able to provide receipts amounting to Rp 1.38. After the increase in soybean prices the value of R / C on total costs decreased to 1.21, meaning that for Rp 1.00 total costs incurred able to provide receipts amounting to Rp 1.21.

## Conclusion

From the results of this study concluded:

1. The increase in soybean prices by 13% (2010-2014) led to price of tofu an increase of 15.97% and has an impact on the decline income of tofu industry amounted to 40.12% or from Rp.2.015.199 be Rp.1.206.765
2. the value of R / C ratio of the industry tofu decreased 12.54% from 1.38 to 1.21. Industry business idea is still in the category feasible to be developed and this led the industry still survive.

## References

[1] Suryana, A. 1990. Diversification of Agriculture in the Process Accelerate the pace of national development. Pustaka Sinar Harapan. Jakarta.

[2] Adisarwanto, T. 2005. Soybean (Raising the Effective Fertilization and Optimization Role foci Roots). Penebar Swadaya. Jakarta

[3] Koswara, S. 2012. Nutritional Value Processing and Preservation Tofu. [On line) Accessed on 25 November 2015.

[4] Sherly. 2013. Effect of Soybean Prices Of Processed Foods Soybeans and Soybean Imports In the Year 2012 [Online]. Accessed on November 25, 2015.

[5] Muchtadi, D. 2009.Principles of Food Processing Technology Source of Protein. Alfabeta. Bandung

[6] Miftahurohman, 2010. Types of Tofu. [Online].https://miftahurrohman01.wordpress.com/jenis-jenis-tahu/Accesed on Januari 2016. Medan

[7] Sarwono, B dan Y. P. Saragih. 2004. Make Aneka Tofu Penebar Swadaya. Jakarta.

[8] Badan Pusat Statistik. 2015.Number in Medan.http://medankota.bps.go.id/ [Online]. Accesed on 2 Februari 2016.
[9] Maryono. 1996.Small Employers: Obstacles faced and empowerment Efforts. Gema Stikubank. Jakarta.
[10] Teguh, M. 2010. Industrial Economics. PT Rajagrafindo Persada. Jakarta
[11] Pasaribu, T.2008. Planning Control Production C, Scientific Journal BEST. Vol 10 No.01 pp 57-65, March 2008

# POSTER PRESENTATION

# ANIMAL SCIENCE

# Total Gas Production, Methane and Rumen Fermentation Characteristics of Rejected Soybean Meal Protected by Jackfruit Leaves

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## Abstract

The research was done to identify total gas production, methane and rumen fermentation product of suplementation of jackfruit leaves on rejected soybean meal. Completely randomized design with three replicated was used in this research. The treatments were: Control (rejected soybean meal); P1 (rejected soybean meal + 0,7% jackfruit leaves) and P2 (rejected soybean meal + 1,4% jackfruit leaves). The 380 mg samples were incubated in 40 ml rumen fluid-buffer for 24 h. Variables measured were total gas production (ml), gas kinetics, $CH_4$ concentration (% total gas production) and rumen fermentation product. Gas production measured at 0, 2, 4, 6, 8, 10 and 24 h time of incubation. Results showed that total gas production, maximum gas production (a+b coefficient) and gas production rate were no significant different. Added the 0,7 and 1,4% jackfruit leaves could reducing 7,61% and 6,22% ($P<0,05$) $CH_4$ production (ml/100 mg organic matter degradability). The conclusion showed that added jackfruit leaves on rejected soybean meal did not affect on total gas production and lower the total VFA value. Added jackfruit leaves contribute positively in reducing $CH_4$. The optimal level of added jackfruit leaves is 0,7%.

Keywords: Jackfruit leaves, Methane, Soybean meal, Total gas production

## 1. Introduction

Feed quality is one of the main factors to improve livestock productivity. The quality of feed depends on include essential ingredients such as energy, protein, fiber, vitamins and minerals. The protein content is the main basis for determining the quality and price of a ruminant feed. Protein is also an important and unique component for ruminants, because the ruminant sources of amino acids derived from two sources, namely feed and rumen microbes

Some source of vegetable protein for ruminants feed such as soybean meal, groundnut cake, coconut cake oil, cotton seed meal and so on. Soybean rejected is an alternative feed ingredients as a source of vegetable protein. This feed is a good source of amino acids (Taghinejad et al., 2009) but their utilization has not been maximized by ruminants. The problem is protein fermentation process conducted by rumen microbes so that high-quality protein digestion are not optimal yet (Ørskov 1982). It also reduces the effectiveness of the use of rich essential amino acids protein in ruminants feed (Busquet et al. 2006). Haryanto (2012) reported that a protein in feed utilization research geared towards making slow deaminated in the rumen protein, so that it can pass through the reticulo-rumen in the intact condition as amino acids.

Slower process of protein degradation in the rumen can be done by using formaldehyde treatment and heating (Hamed & Pasha 2000). The use of antibiotics is also sometimes done to reduce the bacterial population and increase protein fermentation, but it is considered

controversial because it can lead to residues in food products and the influence of genes resistant (Rezaei & Pour 2012). Based on above some alternative research conducted using the plant or plant extracts as natural antibiotics to manipulate rumen fermentation process and improve feed efficiency (Benchaar et al., 2007; Rezaei & Pour 2012). Nature protection protein into rumen by-pass can also be performed using the technique of forming chelate with minerals, tannins protection or use a protective coating with protein material from the rumen fermentation process (Haryanto 2012).

Feed protein protection using tannins that derived from jackfruit leaves are applicable, because this plant thrives in the tropics and ranchers to wear them for ruminant feed mixtures. Another reason is that the jackfruit leaves (leaf + petiole) containing condensed tannins of 130 g / kg dry matter (DM) (Kongmanila & Ledin 2009). In vitro testing is needed to determine the percentage of jackfruit leaf meal provision to protect the protein in soy rejected. Tests using in vitro gas test selected for this method is suitable for screening a single feed ingredients and has a high correlation to the in vivo tests (Menke et al. 1979; Getachew et al., 2004; Hamid et al. 2007). This study aims to determine the total gas production, methane and rumen fermentation products in the soy flour added salvage jackfruit leaves.

## 2. Material and Methods

### *2.1. Material*

The material used in this study are: soybean culled, jackfruit, buffalo rumen fluid, bicarbonate buffer solution, macromineral solution, micromineral solution, resazurin solution, distilled water, and reducing solution. Mediaof buffered rumen fluid made by the method in Krishnamoorthy (2001). Syringe, water bath, thermostat, a series of distillation equipment and MRU gas analyzer was also used in this study.

### *2.2. In vitroIncubation*

Soybean sample rejects and jackfruit leaves are dried in the oven at 60° C, and then pulverized using a grinder to a size of 1 mm. Samples were incubated in vitro using the method of Menke et al. (1979) as modified by Blümmel et al. (1997). A sample of 380 mg were incubated in medium-buffered rumen fluid. Rumen fluid obtained from buffalo berfistula. Buffalo rumen fluid retrieval done in the morning before fed. Rumen fluid filtered through four layers of gauze pads to separate the feed material. Rumen fluid was added to the buffer solution and saturated with CO2 gas for 15 minutes in the tube to maintain anaerobic conditions. Feed samples inserted into the syringe and closed using a piston that has been relubricated with vaseline. A total of 40 ml of media was added to each syringe. The syringe piston is pressed until there are no empty cavity therein and then the initial volume before the samples were incubated recorded. Incubation was performed in a water bath temperature of 39$^{o}$ C.

### *2.3. Design of Experiments and Measurement Parameters*

This study uses a completely randomized design with three replications. The mathematical model of this study is $Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$ where $Y_{ij}$ is the observation on the treatment of all i and replicates all j, μ is the average general, αi is the effect of treatment-th and εij is random influences on factors all i and replicates all j. The data obtained were analyzed using analysis of variance analysis of variance (ANOVA), followed by a further test of Duncan (Matjik & Sumertajaya 2006) with the help of SPSS 16.0 software.

Treatments on this study were;

| | |
|---|---|
| Control | : Rejected soybean meals |
| P1 | : Rejected soybean meals + 0,7% Jackfruit leaves |
| P2 | : Rejected soybean meals + 1,4% Jackfruit leaves |

Nutrient content of treatments are presented in Table 1. The variables observed are the production of gas in total gas production $CH_4$ and fermentation products rumen be a production of $NH_3$, total VFA, dry matter degradation (DDM) and organic matter degradation (DOM). Gas production was observed at 0, 2, 4, 6, 8, 10 and 24 hours of incubation. The reading is done quickly to minimize temperature changes. Calculation of total gas production refers to the procedure in Krishnamoorthy (2001). Kinetics of gas measured by the exponential model Orskov & McDonald (1979) $p=a+b(1-e^{-ct})$. The constants a and b respectively are the soluble fraction and insoluble fraction but can be degraded. The constant c is a constant fraction of the dissolution rate per unit time t. Calculate the fraction of a, b and c using software*fitcurveNaway*®.

$CH_4$ gas production measurement was made after determining the concentration of $CH_4$ gas in total gas fermented each syringe. $CH_4$ concentration was measured using gas MRU Analyzer®. $CH_4$ concentration measurement carried out on the results of incubation accumulated at the 24th hour. Figures are legible at MRU gas Analyzer® is the percentage of $CH_4$ gas is deposited in the syringe. The parameters measured were the concentration of $CH_4$ and $CH_4$ production buffalo rations every 200 mg of organic matter digested. $NH_3$was done based on mikrodifusion conway methods in the General Laboratory Procedure (1966). Total VFA was measured based on steam distillation method. Organic and dry matter degradation measurements and determined according to the calculation fromBlümmel et al. (1997).

## 3. Results and Discussion

### *3.1. Total Gas Production*

The measurement results of treatment of feed gas production is presented in Table 2. It can be seen that the total gas production, gas production maximum (coefficient a + b) and the gas production rate (coefficient c) was not significantly different between treatments. The gas produced in the incubation Hohenheim gas test is derived from the fermentation substrate directly (CO2 and $CH_4$) and comes from gas production indirectly through the mechanism of buffering VFA in the form of CO2 gas is released from the bicarbonate buffer produced during fermentation (Getachew et al. 1998 ; Jayanegara et al. 2009). The addition of 0.7 and 1.4% soy flour jackfruit leaves the salvage has not been proven to lower the total gas production. This proves that the content of tannin in jackfruit leaves not affect the degradation of nutrients in total, but it needs to be viewed from another aspect, namely the production of $CH_4$ (Figure 1) and rumen fermentation products (Table 3).

Tannins protection of jackfruit leaves have not significantly decreased in total gas production of the majority of gaseous $CO_2$ concentration. It can be explained by protein fermentation mechanism will produce ammonia. Ammonia bicarbonate buffer affects equilibrium through the mechanism of neutralization of H + ions without releasing CO2 (Cone and Van Gelder 1999; Salem et al. 2013).

Another reason is that the use of unrefined tannins so there were other nutrients in the leaves of jackfruit. It is precisely to affect and improve feed nutrient degradation. The different results obtained in the study of Mohammadabadi and Chaji (2011), which uses 30 g / kg DM tannin extracted from the leaves of oak and pistachio. In the study explained that the use of tannin extract can decrease total gas production through the mechanism of inhibition of microbial enzymes. Jayanegara et al. (2009) also reported that the addition of low concentration pure tannin (0.5 mg / ml rumen fluid-buffer) can decrease the total gas production in grass hay. The tannins used was derived from extracts of mimosa and quebracho.

Table 1. Nutrient content of feed treatments

| Treatment | DM | Crude Protein | Crude Fat | Ash | NDF | ADF |
|---|---|---|---|---|---|---|
| K | 93,14 | 40,72 | 7,47 | 5,76 | 36,86 | 11,73 |
| P1 | 90,69 | 40,81 | 7,59 | 5,86 | 40,48 | 12,06 |
| P2 | 90,35 | 40,90 | 7,68 | 6,08 | 41,18 | 12,40 |
| SEM | 0,848 | 0.383 | 0.135 | 0,108 | 1,006 | 0,184 |

*K: Rejected soybean meals; P1: Rejected soybean meals+ 0,7% jackfruit leaves meals; P2: Rejected soybean meals+ 1,4% jackfruit leaves meals; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre; SEM: Standard Error of Mean.*

Table 2. Total gas production, a+b and c after 24 hours Incubation

| Perlakuan | Waktu koleksi (jam) | | | | | | Kinetika gas | |
|---|---|---|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | 10 | 24 | a+b | c |
| K | 10,05 | 21,78 | 30,61 | 38,51 | 44,23 | 66,17 | 73,51 | 0,098 |
| P1 | 9,52 | 19,28 | 27,56 | 34,74 | 40,11 | 64,36 | 74,12 | 0,095 |
| P2 | 10,59 | 22,68 | 32,39 | 40,12 | 45,30 | 67,59 | 74,32 | 0,101 |
| SEM | 0.390 | 0.905 | 1,099 | 1,272 | 1,373 | 0.849 | 2,164 | 0.006 |

*K: Rejected soybean meals; P1: Rejected soybean meals+ 0,7% jackfruit leaves meals; P2: Rejected soybean meals+ 1,4% jackfruit leaves meals; a+b: Maximum gas production; c: Gas production rate; SEM (standard error mean).*

### *3.2. Concentration and $CH_4$ Gas Production*

The increase in total gas production is followed by increased production of $CH_4$. The higher total gas production will lead to the production of $CH_4$ gas is high. This led to the assessment of emission reductions $CH_4$ using variable concentrations of $CH_4$. $CH_4$ concentration results feed incubation treatment for 24 hours and $CH_4$ production per 100 mg of undigested organic matter is presented in Figure 1. The addition of jackfruit leaves 0.7% and 1.4% may decrease the concentration of $CH_4$ respectively of 3.50% and 5 , 66% ($P < 0.05$). In addition the same level, jackfruit leaves also can lower $CH_4$ gas production (ml / 100 mg ingested BO) amounted to 7.61% and 6.22% ($P < 0.05$).

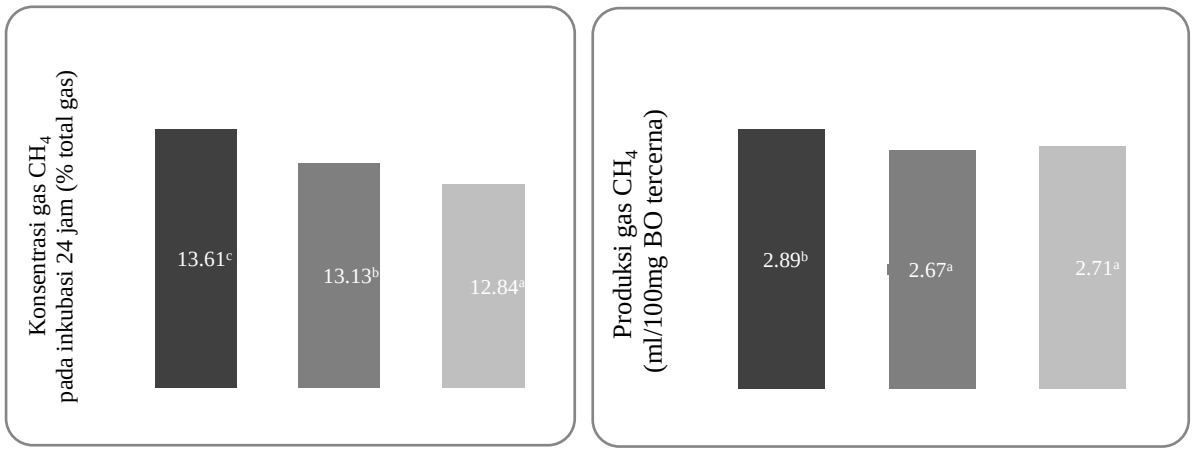


Figure 1. The concentration of $CH_4$ (left) and $CH_4$ gas production (right) in each 100 mg of organic matter digested feed incubated for 24 hours of treatment. K: Rejected soybean meals; P1: Rejected soybean meals + 0,7% jackfruit leaves meals; P2: Rejected soybean meals + 1,4% jackfruit leaves meals); Different superscripts indicate significant differences ($P < 0.05$).

Reduction of concentration and tannin production due to the addition of jackfruit leaves are important because $CH_4$ gas production decreased to reduce the energy loss of feed is wasted, thereby increasing the efficiency of feed utilization by livestock (Widiawati et al. 2010). Improved efficiency can be observed in decreasing the concentration of the gas due to the provision of jackfruit leaves (Table 2) without affecting the cumulative gas production (Table 2).

$CH_4$ gas reductions are the result of the interaction of condensed tannins in the leaves of jackfruit. Mixed leaves and petioles in jackfruit leaves contain condensed tannins at 130g / kg dry matter (DM) (Kongmanila and Ledin 2009). Cortes et al. (2009) explains that the tannin has a strong tie to the protein so that it can provide protection from degradation by rumen microbes. Elements of proteins in microbial enzyme will be bound by the use of tannins. Jayanegara et al. (2009) also explained that the tannins are not only interact with the protein, but also against the fiber and other components such as vitamins and minerals. CH4 gas reductions can also be caused by mechanisms tannins inhibit the growth of protozoa. Protozoa are one of the main host methanogenic bacteria (Goel et al., 2008). Condensed tannins or proanthocyanidins are polymers of the flavonoid molecules that function as anti-microbial (Bodas et al. 2012). In some studies have also explained that the tannins contribute to inhibit the growth of cellulolytic and proteolytic bacteria (Mc Sweeney et al. 2001; Bodas et al. 2012). It makes use of tannin concentration must be regulated so as not to negatively affect the digestion and absorption of nutrients.

*3.3. Rumen Fermentation Products*

Rumen fermentation product is observed pH, NH3, VFA total, DDM and DOM produced after 48 hours of incubation (Table 3). Variable pH, NH3 and DDM were not significantly different between treatments. In total VFA variables shown that the addition of jackfruit leaves can reduce total VFA production ($P < 0.05$). The addition of jackfruit leaves can also increase the value of DOM ($P < 0.05$). Variable pH of the three treatments are still in the normal range is 5.5 to 7.2 (Owens &Goestsch 1988). It is proved that the administration of 0.7 and 1.4% of jackfruit leaves no negative effect on the rumen ecosystem. At normal pH / stable, rumen fermentation processes can run optimally. The addition of jackfruit leaves did not leave a noticeable effect on the concentration of NH3. This is caused by the content of which is identical among the treatments PK feed (Table 1). The amount of dietary protein is one of the factors that affect the production of NH3 (McDonald et al. 2002). NH3 concentration value of the three treatments ranged from 26.67 to 33.33 mg / 100 ml. Normal concentrations for microbial fermentation in the culture of a closed system is 5 mg / 100 ml, but depending on the level of feed Fermentability (Wanapat&Rowlison 2007; Wanapat et al. 2013). NH3 concentration is higher in the three treatments of feed caused by the accumulation of NH3 production during 48 hours of incubation. Accumulation caused by the lack of uptake of the product during fermentation in a closed system.

The addition of jackfruit leaves on a 0.7% soy flour rejects lowering the total VFA production by 29% ($P < 0.05$). The addition of 1.4% jackfruit (P2) also can reduce total VFA production, but not significantly different when compared with P1. The decline in total VFA production thought to be caused by protozoa population decline due to the mechanism of tannins inhibit the growth of protozoa. Tannins have a strong holding capacity of the protein so that it can provide protection from degradation by rumen microbes (Cortes et al., 2009).

Tabel 3. Rumen fermentation products feed inkubatedfor 24 hours treatment.

| Treatment | pH | $NH_3$ (mg/100ml) | VFA total (mM) | DDM (%) | DOM (%) |
|---|---|---|---|---|---|
| K | 6,99 | 26,67 | 66,67[b] | 87,33 | 88,67[a] |
| P1 | 7.03 | 33,33 | 47,33[a] | 91,90 | 94,39[b] |
| P2 | 7.09 | 26,67 | 54,67[a] | 89,94 | 94,86[b] |
| SEM | 0,061 | 3,093 | 3,027 | 1,046 | 1,214 |

*K: Rejected soybean meals; P1: Rejected soybean meals + 0,7% jackfruit leaves meals; P2: Rejected soybean meals + 1,4% jackfruit leaves meals); $NH_3$: amonia; VFA: volatile fatty acid; DDM: Degradasi Dry Matter; DOM: Degradasi organic matter. Different superscripts indicate significant differences (P <0.05).*

VFA concentration change is a reflection of changes in rumen microbial population (Pamungkas et al. 2006). Something similar is obtained in the study El-Waziry et al. (2007) reported that the VFA production declined significantly in samples of soy flour were added pure tannin. Jayanegara et al. (2009) also reported that the addition of 0.5 mg / ml of pure tannin on the substrate hay can reduce total VFA production by 5.7 to 11.7%.

The decline in total VFA production is proportional to the decrease in production of CH4 (Figure 1). It is proved that the ecosystem changes rumen protozoa population as a decrease associated with a decrease in the population of methanogenic bacteria. Protozoa are one of the main host methanogenic bacteria (Goel et al., 2008). Production of total VFA third-feed treatment ranged from 47.33 to 66.67 mM. In Chanthakhoum research and Wanapat (2012) reported that the rumen buffaloes fed rice straw, rice straw and concentrate fermentation produces total VFA production respectively 44.8; 48.9 and 55.9 mM (Chanthakhoun&Wanapat 2012). VFA production total on a single substrate, written hay pure tannin low concentrations ranged from 40.6 to 46.0 mM (Jayanegara et al. 2009).

DDM is not visible in the variable effect of the addition of jackfruit leaves the soy flour rejected. This represents a decrease of microbial activity in producing the VFA does not affect the degradation of the feed (in BK). This contrasts with research Mohammadabadi and Chaji (2011) reported that the administration of fruit extract tannins and oak leaves pistachios may lower the level of degradation of the feed substrate in the medium in vitro. The interesting thing is there is an increase in soy flour salvage DOM added jackfruit leaves (Table 3). This is thought to occur because of two reasons: 1) The use of tannins contained in jackfruit without going through the extraction process first. This resulted in the inhibition mechanism of microbial activity is not optimal; and 2) The interaction of nutrients is still contained in the leaves of jackfruit. It will actually be used by microbes to proliferate. Still the microbial activity is represented by the concentration of ammonia and total VFA production in the normal range throughout the study (Table 3). The efficiency of microbial growth is influenced by the availability and balance of protein and carbohydrates in the rumen (Leng 1991).

## Conclusion

Addition ofjackfruit leaves at the level of 0.7% and 1.4% did not significantly affect the total gas production in rejected soybean meals. Tannin content in jackfruit leaves also negatively affect rumen digestion process is represented by a decline in total VFA content. However, the provision of jackfruit leaves also contribute positively to the reduction of $CH_4$ gas production so as to improve the efficiency of the use of rejected soybean meals as ruminant feed.

Addition optimum jackfruit leaves are at the level of 0.7%.

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## References

Benchaar C, Petit HV, Berthiaume R,Ouellet DR,Chiquette J, Chouinard PY. 2007. Effects of essential oils on digestion, ruminal fermentation, rumen microbial populations, milk production and milk composition in dairy cows fed alfalfa silage or corn silage.J. Dairy Sci. 90:886–897.

Blümmel M, Makkar HPS, Becker K. 1997. The in vitro gas production: A technique revisited. J.Anim. Phys. Nutr. 77: 24–34.

Bodas R, Prieto N,Garcia-Gonzalez R,Andres S, Giraldez FJ, Lopez S. 2012. Manipulation of rumen fermentation and methane production with plant secondary metabolites. Anim. Feed Sci. Tech. 176: 78– 93.

Busquet M,Calsamiglia S, Ferret A, Kamel C. 2006. Plant extracts affect *in vitro* rumen microbial population.J. Dairy Sci. 89: 761-771.

Chanthakhoun V, WanapatM. 2012. The in vitro gas production and ruminal fermentation of various feeds using rumen liquor from swamp buffalo and cattle. Asian J.Anim. Vet. Adv. 7 (1): 54-60.

Cone JW, Van Gelder AH. 1999. Influence of protein fermentation on gas production profiles. Anim. FeedSci. Tech.76: 251-264.

Cortes JE, Morenob B, Pabón ML, Avila P, Kreuzerd M, Hess HD, Carulla JE. 2009. Effects of purified condensed tannins extractedfrom Calliandra, Flemingia and Leucaena on ruminal andpostruminal degradation of soybean meal as estimated *in vitro*.Anim. Feed Sci.Technol.151: 194–204.

El-WaziryAM, Nasser MEA, Sallam SMA, Abdallah AL, Bueno ICS. 2007. Processing methods of soybean meal, 2. Effect of autoclaving and Qucbraho tannin treatedsoybean meal on gasproduction and rumen fermentation *in vitro*. J. Appl.Sci. 3: 17-24.

General Laboratory Procedure. 1966. Department of Dairy Sciences. Madison: University of Wisconsin.

Getachew G, Blümmel M, Makkar HPS, Becker K. 1998. *In vitro*gas measuring techniques for assessment of nutritional quality of feeds: a review. Anim. Feed Sci. Tech. 72: 261-281.

Getachew G, Makkar HPS, Becker K. 2000. Effect of polyethylene glycol on *in vitro*degradability and microbial protein synthesis fromtannin-rich browse and herbaceous legumes. B. J.Nutr. 84: 73-83.

Goel G, Makkar HPS, Becker K. 2008. Effect of *Sesbania sesban* and *Carduus pycnocephalus* and Fenugreek (*Trigonella foenum-graecum* L.) seeds and their extracts on partitioning of nutrients from roughage-andconcentrate-based feeds to methane. Anim.Feed Sci. Technol. 147: 72-89.

Hameed F, Pasha TN. 2000. Effect of varying levels of formaldehyde and heat treatmenton *in situ* ruminal degradation of different vegetable proteinmeals. Int. J. Agric. Biol. 2 (1-2): 48-51.

Hamid P, Akbar T, Hossein J, Ali MG. 2007. Nutrient digestibility and gas production of some tropical feeds used in ruminant dietsestimated by the *in vivo* and *in vitro* gas production techniques. Am. J. Anim. Vet. Sci. 2 (4): 108-113.

Haryanto B. 2012. Perkembangan penelitian nutrisi ruminansia. Wartazoa. 22 (4): 169-177.

Jayanegara A. Makkar HPS, Becker K. 2009. Emisi metana dan fermentasi rumen *in vitro* ransum hay yang mengandung tannin murni pada konsentrasi rendah. Media Peternakan 32(3): 185-195.

Kongmanila D, Ledin I. 2009. Chemical Composition of Some Tropical Foliage Species and Their Intake and Digestibility by Goats. Asian-Aust. J. Anim. Sci. 22 (6): 803-811.

Krishnamoorthy U. 2001. RCA Training Workshop on In Vitro Techniques for Feed Evaluation. The International Atomic Energy Agency. Jakarta, April 23-27th 2001: 17.

Leng RA. 1991. Application of Biotechnology to Nutritionof Animals In DevelopingCountries. FAO Animal Production and Health Paper 90. Rome.

Mattjik AA, Sumertajaya M. 2006. Perancangan Percobaan dan Aplikasi SAS dan Minitab. Jilid I. Edisike 2. Bogor (ID): IPB Press.

McDonald P, Edward RA, Greenhalgh JFD, Morgan CA. 2002. Animal Nutrition. 6th Edition. New York (NY): Scientific and Tech John Willey & Sons. Inc.

McSweeney C, Palmer B, Bunch R, Krause D. 2001. Effect of the tropical forage calliandra on microbial protein synthesis and ecology in the rumen. J. Appl. Microbiol. 90: 78–88.

Menke KH, Raab L, Salewski A, Steingass H, Fritz D, Schneider W, 1979. The estimation ofthe digestibility and metabolizable energy contentof ruminant feeding stuffs fromthe gas production when they are

incubated with rumen liquor. J.Agric. Sci. 93: 217-222.

Mohammadabadi T, Chaji M. 2012. The influence of the plant tannins on *in vitro* ruminal degradation and improving nutritivevalue of sunflower meal in ruminants. Pak. Vet. J. 32(2): 225-228.

Ørskov ER, Mcdonald I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to the rate of passage.J. Agr. Sci. Camb. 92: 499-503.

Ørskov ER. 1982. Protein Nutrition in Ruminants. Academic Press, London. England.

Owens FN, Goetsch AL. 1988. Ruminal fermentation. In.D.C. Church (Ed). The Ruminant Digestibility Physiology and Nutrition. Prentice Hall, New Jersey. pp. 145-171.

Pamungkas D, Sevilla CC, Lustria UM. 2006. Changes in rumen ecosystem and feed dry matter degradability of buffalo which received rumen content of cattle through cross inoculation. JITV. 11 (1): 24-33.

Rezaei N, Pour HA. 2012. Evaluation effect of thyme extract on degradability of soybeanmeal with gas product technique. Adv.Biores.3 (3): 45 – 47.

Salem AZM, Chuan-She Z, Zhi-liang T, Mellado, Salazar MC, Elghandopur MMMY, Edongo NE. 2013. In vitroRuminal Gas Production Kinetics of Four Fodder Trees Ensiled With or Without Molasses and Urea. J. Integr. Agr. 12(7): 1234-1242.

Taghinejad M, Nikkhah A, Sadeghi AA, RaisaliG, Chamani M.2009. Effects of gammairradiation on chemical composition,antinutritional factors, ruminal degradation and *in vitro* protein digestibility of full-fat soybean. Asian-Aust. J. Anim. Sci.22 (4): 534–541.

Wanapat M, Kang S, Phesatcha K. 2013.Enhancing buffalo production efficiency through rumen manipulation and nutrition. Buffalo Bull. 32 (1): 258-275.

Wanapat M, Rowlinson P. 2007. Nutrition and feeding of swamp buffalo: feed resources and rumen approach. Ital. J. Anim. Sci. 6 (Suppl. 2): 67-73.

Widiawati Y, Winugroho M, Mahyuddin P. 2010. Estimasi produksi gas metana dari rumput dan tanaman leguminosa yang diukur secara in vitro. Prosiding seminar nasional teknologi peternakan dan veteriner 3-4 Agustus 2010. Bogor: 131-136.

# The Supplementation of Amino Acid Methionine-Lysine on the Protein Quality of Leucaena Leaf Meal Fermented with *Bacillus laterosporus*

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## Abstract

This study aimed to determine the level of provision of optimal amino acid methionine-lysine in fermentation leucaena leaf meal (LLM) with *Bacillus laterosporus* for the preparation of ration ducks. This study used a completely randomized design with three replications. Each treatment consists of A = LLM fermentation with *Bacillus laterosporus*+0% supplement amino acid methionine-lysine (control), B = A+0.25% methionine+0.50% lysine, C = A+0.30% methionine+0.75% lysine, D = A+0.35% methionine+1.00% lysine and E = A+0.40% methionine+1.50% lysine. Parameters measured were amino acids methionine and lysine after methionine-lysine supplementation. The result indicated that the content of the amino acid methionine for the addition of amino acid supplements methionine-lysine was not significant ($P>0.5$) but significant ($P<0.05$) the content of the amino acid lysine. It can be concluded that supplementation of amino acid methionine 0.40%+1.50% lysine to LLM fermentation with *Bacillus laterosporus* gives the best results.

Keywords: supplementation, amino acid, fermentation, *Bacillus laterosporus*

## 1. Introduction

The content of nutrients produced in the fermentation process, especially protein increases, it is caused by bacteria / microorganism during fermentation containing single cell protein and also produce enzymes that count as protein. On the other hand the product of fermentation produces the nucleic acid that is non protein nitrogen (NPN) which is not a protein so that the use in poultry should be tested to determine whether the amino acids of proteins containing amino acids are good [1].

By products of fermentation is a nucleic acid which is a protein that contains NPN where proteins are not utilized optimally in poultry because it does not have the enzyme ribonuclease and will be discarded along with the feces, so that the resulting protein is not a protein-containing amino acids are complete. Then added [2] that Leucaena deficiency in amino acids methionine and lysine, to be used optimally need additional methionine amino acid lysine synthesis.

A protein quality feed ingredient, among others, determined by the completeness and balance of essential amino acids contained therein. High-quality proteins usually contain essential amino acids complete, the numbers are sufficient and balanced. The preparation of poultry rations is now the focus of attention is no longer on the amount of protein that must be provided, but more attention to the balance between the energy of the essential amino acids, because the essential amino acids can not be

synthesized in the body [3], so it needs to be supplied in the ration is consumed by the addition of amino acid synthesis.

Amino acids are the fundamental building blocks of protein. About 22 different amino acids contained in the proteins of the body. All that is not to be available in poultry rations and can be synthesized in the body. but the amino acids are the following: lysine, arginine, histidine, leucine, isoleucine, valine, methionine, threonine, phenylalanine tryptophan and its presence in the diet is absolutely necessary because the duck can not synthesize in the body, it is classified as ten amino acid essential [4].

Methionine is one of the essential amino acids, therefore must be provided in the diet in sufficient quantities, in addition to the amino acid methionine is a major barrier in chicken rations [5]. Furthermore, [6] suggests that the methionine is a substance that is essential for poultry, which is in line with the statement [7] and [8], that the establishment of the breast meat in broilers is very sensitive influenced by methionine in the rations.

Lysine which has many uses in the body is an amino acid that cannot be synthesized by the body of the chicken, so classified in essential amino acids are critical for very low levels in the feed. Due to the lack of essential amino acids in feed ingredients, then chicken rations need to be supplemented with synthetic lysine amino acid in accordance with the needs of the poultry [9].

Furthermore, in preparing the feed of poultry is highly considered amino acids methionine-lysine, because amino acids are essential amino acids and are also called amino acids barrier that must be brought in from outside because the poultry can notproduce it themselves, an amino acid that is not derived from plant materials but from the animal. Methionine is an amino acid deficiency-lysine of vegetable can also be met by the addition of methionine-lysine synthesis. Thus will lysine methionine amino acid supplementation on quality of leucaena leaf meal fermented with *Bacillus laterosporus* needs to be done. It is expected to help farmers reduce the use of fish meal and soybean meal in poultry rations.

## 2. Material and Methods

The study was conducted at the Integreted Laboratory of Kopertis Region X Padang and the Integrated Laboratory of IPB Bogor and the Center For Post Harvest Developmentin Bogor, Year of 2016. The studies include the enrichment of the bacteria to gain rejuvenation and manufacture of inoculum fermentation bacteria (*Bacillus laterosporus*) as well as the manufacture of products leucaena leaf meal (LLM) fermented with *Bacillus laterosporus* without the addition of amino acid supplements-lysine and methionine plus supplemental amino acids methionine-lysine by using a complete randomized design (CRD), 5 treatments with 3 replications. Parameters measured were amino acids methionine, lysine without and after supplementation of amino acids lysine and methionine and also *betacarotene* content.

The composition of the treatment is:

A = LLM Ferm *Bac. Laterosporus* without supplement met-lysine(as control)
B = LLM Ferm *Bac. laterosporus* + 0,25% and 0,50% supplement met-lysine
C = LLM Ferm *Bac. laterosporus* + 0,30% and 0,75% supplement met-lysine
D = LLM Ferm *Bac. laterosporus* + 0,35% and 1,00% supplement met-lysine
E = LLM Ferm *Bac. laterosporus* + 0,40% and 1,25% supplement met-lysine

Results of analysis of variance included in the table to determine the effect of treatment. Test of DMNRT at the level of 5% was used to compare between treatments. Data was analyzed according to the procedures of [10].

### *2.1. Implementation Trial*

Leucaena leaf meal fermentation process with *Bacillus laterosporus*, according to the following procedure:

1. Substrate preparation leucaena leaf Leucaena.
   Leucaena used in this study, is a local leucaena that have a high 2-5 m, located was obtained around the City of Padang, West Sumatra, the leaves are taken, then the oven temperature of $60^0$C for 24 hours, then milled to be used as LLM.
2. Preparation of inoculums
   Making inoculum using substrates bran 100 g plus 60 ml of distilled water in the autoclave for 30 minutes at a temperature of $120^o$C, 1 atm and then cooled to a temperature of about 37 °C. Taken tube containing *isolate*, add 20 ml of distilled water, then crushed loopful. Furthermore then in vortex so that a homogeneous solution, mix into a plastic isolate containing bran, stirring until evenly distributed and closed then a hole to keep the aeration. Incubated for 24 hours at a temperature of $37^0$C.
3. Procedure of fermentation
   Dry substrate LLM weighed with a weight of 1 kg. Added 800 ml of distilled water. Then autoclaved for 30 minutes at a temperature of $121^0$C, 1 atm. After that inoculated with *Bacillus laterosporus* as much as 6% of the amount of substrate, then incubated for 24 hours [11], so the fermented product is dried at $60^0$C for 24 hours. The dried product ready for use. The process of making meal products leucaena leaf meal are fermented *Bacillus laterosporus* for amino acids and *betacarotene* analysis, the best results are used for further research.

The process of making leucaena leaf meal of product fermented with *Bacillus laterosporus* which can be seen in Fig. 1.

*2.2 Targeted Results:*

1. Getting the best level LLM fermented with *Bacillus laterosporus* with a dose of 6% and fermentation time 24 hours, the best before and after supplementation be given lysine amino acid methionine.
2. Getting the analysis of amino acids methionine and lysine fermentation leucaena leaf meal best products before and after the supplements of amino acids methionine-lysine.

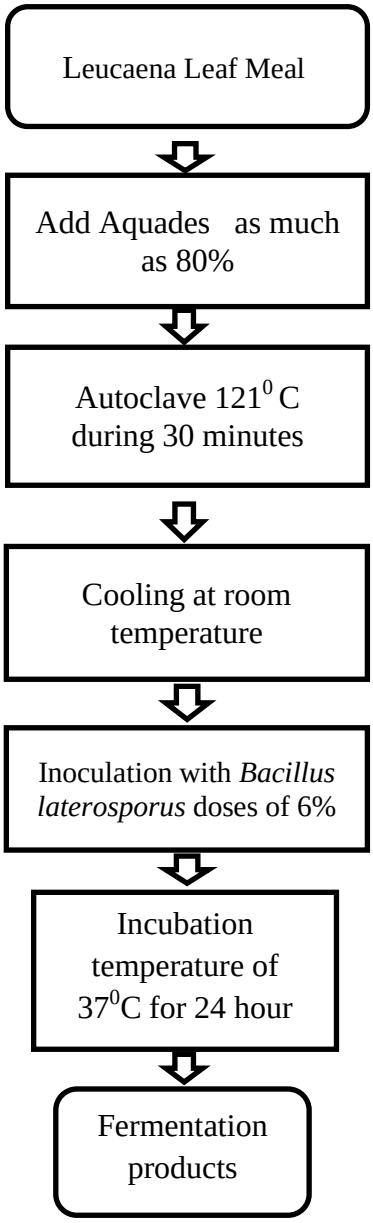


Fig. 1 Making procedures LLM fermentation with *Bacillus laterosporus* (modified from [12]).

## 3. Result and Discussion

### *3.1. Effect of Amino Acid Supplementation on Methionine-lysine content ofMethionine Amino Acid Fermentation Products*

Methionine amino acid content of LLM fermentation products methionine-lysine supplements given during the study in Table 1. Results of variance showed that the level of supplementation of amino acids Methionine-lysine to methionine amino acid content of LLM fermentation products provide no significant effect (P> 0.05).

The results in Table 1 showed that the level of supplementation with the amino acid methionine-lysine to the amino acids methionine fermented leucaena leaf meal (LLM) showed that the treatment E (Extrasupplement amino acids 0.40% methionine + 1.25% lysine) increased content the amino acid methionine highest compared with other treatments is 0.30% and the value is better than the results of the control that is 0.183%.

Described by the [13], [14] and [15] that methionine is an amino acid superior to other amino acids in the increased weight of the eggs, as an amino acid synthetic in the form of a mixture of DL-methionine acts as a donor methyl, utilization in the form of isomer 100%, so it plays a role in helping other metabolism in the body such as metabolism choline, protein and carbohydrates. Added [16] required the addition of amino acids methionine 0.1%-0.2% in the ration to increase egg weight and high usage efficiency ration.

### *3.2. Effect of amino acid supplementation on methionine-lysine content of lysine amino acid fermentation products.*

Lysine amino acid content of leucaena leaf meal fermentation products methionine-lysine supplements given during the study in Table 2.

Table 1. Mean Content of Amino Acids Methionine LLM Fermentation Given Supplements Methionine-Lysine for Research.

| Threatment | The Content of Metionin (%) |
|---|---|
| A = LLM ferm without treatment | 0,183[a] |
| B = LLM ferm*Bac. laterosporus* + 0.25% methionine + 0.50% lysine | 0,200[a] |
| C = LLM ferm*Bac. laterosporus* + 0.30% methionine + 0.75% lysine | 0,207[a] |
| D = LLM ferm*Bac. laterosporus* + 0.35% methionine + 1.00% lysine | 0,208[a] |
| E = LLM ferm*Bac. laterosporus* + 0.40% methionine + 1.25% lysine | 0,300[a] |

The numbers followed by the same letter are not significantly different according to the test of DNMRT on a real level 5%

Table 2. Mean Content of Amino Acids Lysine LLM Fermentation Given Supplements Methionine-Lysine for Research.

| Threatment | The Content of Lysine (%) |
|---|---|
| A = LLM ferm without treatment | 0.873[a] |
| B = LLM ferm*Bac. laterosporus* + 0.25% methionine + 0.50% lysine | 0.993[ab] |
| C = LLM ferm*Bac. laterosporus* + 0.30% methionine + 0.75% lysine | 1.193[ab] |
| D = LLM ferm*Bac. laterosporus* + 0.35% methionine + 1.00% lysine | 1.063[ab] |
| E = LLM ferm*Bac. laterosporus* + 0.40% methionine + 1.25% lysine | 1.353[b] |

The numbers followed by the same letter are not significantly different according to the test of DNMRT on a real level 5%

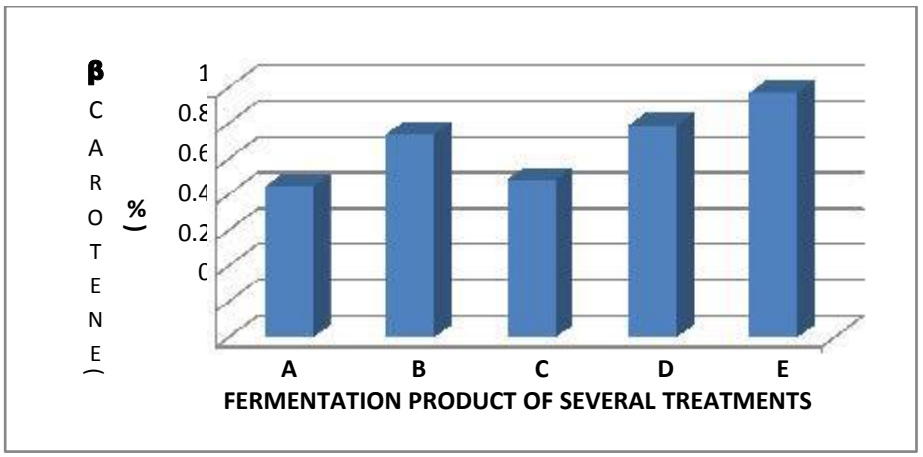


Fig. 2 *Betacarotene* content of fermentation product in several treatments

Results of variance showed that the level of supplementation of amino acids methionine-lysine to the amino acids lysine of LLM fermentation products provide a significantly different effect ($P < 0.05$).

The results in Table 2 showed that the level of supplementation with the amino acid methionine-lysine to the amino acids lysine fermented of LLM showed that the treatment E (Extra supplement amino acids 0.40% methionine + 1.25% lysine) increased content the amino acid lysine highest compared with other treatments is 1.353% and the value is better than the results of the control that is 0.873%.

Described by [17] and [9] that lysine which have many uses in the body is an amino acid that can not be synthesized by the body of the chicken, so classified in essential amino acids essential for very low levels in the feed. Lysine produce energy inhibits the formation of fat. Due to the lack of essential amino acids in feed ingredients, then chicken rations need to be supplemented with synthetic lysine amino acid in accordance with the needs of livestock. Furthermore, [18] states that the supplementation of the amino acid methionine (0.47%) and lysine (1.1%) with a protein content of 15% can increase the performance of crossbred Mojosari-Alabio ducks.

### *3.3. Effect of amino acid supplementation on methionine-lysine content of betacarotene fermentation products.*

*Betacarotene* content of leucaena leaf meal fermentation products with the Methionine-lysine can be seen in bar chart on Fig. 2.

*Betacarotene* is a carotenoid group unstable and easily oxygenated become *xanthophyl* and *xanthophyl* must come from outside because the poultry are not able to synthesize [19] and [20]. Obtained from the treatment accorded treatment supplementation of amino acids methionine 0.40% + 1.25% lysine gives the best results *betacarotene* namely 68, 49%.

## Conclusion

The research results can be concluded that treatment of fermentation obtained leucaena leaf meal (LLM) with supplementation of amino acids methionine and lysine to the level of 0.40% methionine and 1.25% lysine are best able to improve the content of 24.22% methionine and 21.56% lysine compared with no treatment (control).

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## References

[1] Scott, M.L., M.C Nesheimdan R.J Young. *Nutrition and management of ducks*. M.I. Scott of Ithaca, Ithaca, NY. pp: 74-78. 1991.

[2] Laconi, E.B. dan T. Widiyastuti. KandunganXanthofildaunLeucaena (*Leucaenaleucochepala*) hasildetoksifikasiMimosinsecarafisikdankimia. Jurnal Media Peternakan. ISSN 0126 – 0472. Vol. 33 No. 1. Hlm : 50 – 54. 2010. (in Indonesia)

[3] Aisjah, T., R. Wiradimadja dan Abun. *Suplementasi Metionin dalam ransum berbasis lokal terhadap imbangan efisiensi protein pada ayam pedaging*. Laporan penelitian. Jurusan Nutrisi dan Makanan Ternak, Fakultas Peternakan, Universitas Padjajaran, Bandung.2007. (in Indonesia)

[4] National Research Council [NRC]. *Nutrient Requirement of Poultry*. National Academy Press, Washington, D.C. USA. 1994.

[5] Weerden, E.J., J.B. Schutte and H.L. Bertran.. Comparison of D.L. Methionine, D.L. Methionine Analogue Free Acid With Layers. Poultry Sci. 63 : 1793 – 1799. 1984.

[6] Schutte, J.B. J. De Jong, W. Smink, and M. Pack. ReplacementValue of Betaine for D.L. Methionine in Male Broiler Chicks. Poultry Sci. 76 : 321-325. 1997.

[7] Huyghebaert, G., M. Pack and G. de Groote. Influence of Protein Concentration on the Response of Broilers to Supplemental D.L. Methionine. Arch. Gefliigelhd. 58 (1) : 23-29. 1994.

[8] Schutte, J.B., and M. Pack. Sulfur Amino Acid Requirement of Broiler Chicks. From Fourteen to Thirty Eight of Age: I Performance and Carcass Yield. Poultry Sci. 74 : 480 – 487. 1995.

[9] Anggorodi, R. 1995. *Nutrisi Aneka TernakUnggas*. GramediaPustakaUtama, Jakarta.(in Indonesia)

[10] Steel, R. G. D. dan J. H. Torie. *Prinsip dan Prosedur Statistika. Suatu Pendekatan Iometrik*. PT. Gramedia Pustaka Utama, Jakarta. 1995. (in Indonesia)

[11] Yessirita, N., H. Abbas., Y. Heryandidan A. Dharma. Effect of dose and time of leaf Leucaena (*leucaenaleucocephala*) Fermentation with Bacillus laterosporus to dry Mmatter, crude protein and crude fiber. Proceeding in International Seminae 1st Indonesian Poultry Science Seminar on September 11 – 12, p: 367 – 372 . 2012. Faculty of Animal Science, University of Andalas, Padang, West Sumatera, Indonesia, in Conjunction with WPSA Indonesia Branch.

[12] Fardiaz, S. *Penuntun Praktek Mikrobiologi Pangan*. Penerbit IPB, Bogor. 1989. (in Indonesia)

[13] Leeson, S dan J. D. Summers. *Comercial Poultry Nutrition*. Thirth Edition. Departement of Animal and Poultry Science. University of Guelph Ontariom, Canada. 2001.

[14] Safaa, H.M., D.G. Valencia., E. Arbe., E. Jibenez-Morena., R. Lazaro., G.G. Moteos G.G. Effect of the level of Methionine, linoleic acid and added fat in the diet on productive poerformance and egg quality on Brown laying hen the late phaseroduction. Poult. Sci. 87 (8): 1595-602. 2008.

[15] Keshavarz,K. Effects of reducing dietary protein, Methionine, Choline, Folic Acid, and Vitamin B12 During the late stages of the egg production Cycle on Performance and Eggshell Quality. *Poultry Science*82 : 1407 – 1414. 2003.

[16] Shen, T.F. Nutrient Requirement of Egg-Laying Duck in : *Duck Production Science and World Practice*. Farrel, D. J and Starpeleton (Ed). Univ of New England. Indarmidale. 1985.

[17] Zainuddin, D. PenentuanKebutuhanAsam Amino danEnergiMetabolisuntukproduksiTelurAyamTipe Medium di Daerah Tropis. Disertasi. FakultasPascaSarjanaInstitutPertanian Bogor, Bogor. 1990. (in Indonesia)

[18] Silitonga, L. Supplementasiasam amino LisindanMetioninsintesiadalamransumdaribahannabatiterhadapperformansitikpersilanganMojosari Alabio. Thesis. Program Pascasarjana, IPB, Bogor. 2003. (in Indonesia)

[19] Hausmann, A and G. Sandmann. A single five-step desaturase is involved in the carotenoid biosynthetis pathway to beta-carotene and torulene in *Neusporacrassa*. J. Genet. Biol. 30(2): 147-53. 2000.

[20] Fenita, Y., U. Santoso, H. Prakoso. Pengaruh suplemen Metionin Lisin dan Triptophan dalam ransum berbasis lumpur sawit fermentasi terhadap performa, produksi dan kualitas telur ayam ras. Jurnal Sains Peternakan Indonesia. Vol.5(2): 105-114. 2010. (in Indonesia)

# AGRICULTURE

# Optimization of Koji Concentration And Fermentation Time to Characteristics of Modified Sorgum (*Sorghum Bicolor L. Monench*) Flour

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## Abstract

Optimization of koji concentration and fermentation time in the manufacture of modified sorghum flour has been done. The purpose of this research is to inform the processing of fermented sorghum flour modified by using koji and its influence on fermentation time where the sorghum flour as a substitute flour can be processed into various products such as bread, cookies, noodles and some extrusion products. This research uses a split plot design with a 3x5 factorial design. The study was conducted to determine the effect of fermentation time is carried out for 24 hours, 36 hours and 48 hours and koji concentration of 2%, 4%, 6%, 8% and 10% on the characteristics of sorghum flour modified fermentation. Chemical analysis carried out on water content (gravimetric method), starch and dextrin levels using luff schoorl method and organoleptic tests include color and aroma by using hedonic preference test. The analysis showed that the fermentation time factor effect on water content, starch and dextrin. Koji concentration factors affect the water content, starch and dextrin, and there was an interaction between fermentation time and concentration of koji to moisture, starch and dextrin modified sorghum flour. The best products produced on fermentation time of 48 hours and a concentration of 10% koji which has a moisture content of 5.65%, 26.73% starch, dextrin content of 13.02%.

Keywords: grain sorghum, koji concentration, fermentation time, modified sorghum flour

## 1. Introduction

Generally, Indonesian people make rice as a main food source of calories, this predictable pattern of eating less than ideal that would pose a risk to the health and food security, because in terms of the balance of the food, especially the balance of carbohydrates, the diet of people Indonesia is considered less than ideal due to the high consumption of rice as one The only source of calories. Processing of cereals is one strategic step in providing food support food diversification program. Carbohydrate source that grows in Indonesia of various types, but are cultivated intensively to meet the caloric needs of Indonesian society is still limited to rice and corn (Herry et al, 2010).

The problem of domestic food can not be separated from the issue. Whole wheat and rice flour needs met from imports, namely for bread 20%, 50% noodles, biscuits and snacks (snacks) 10% and the rest for domestic use. Currently the national flour needs to reach 5 million tons/year, even in 2009 nearly 6 million tons/year. If this condition continues it will threaten the food security and food sovereignty. Therefore flour utilization of local raw materials need to be developed (Richana et al, 2010).

Answering these problems, the government implemented a program of diversification, but its implementation is still not produce the expected changes in diet. Diversification of food consumption with diverse sources of carbohydrate, protein, fat,

vitamins and minerals is still less than optimal because of the limited production of diversified agri-food commodities. Cereals such as sorghum is a commodity with a potential, not only as a source of carbohydrates, but also as a source of antioxidants, bioactive compounds and dietary fiber that are important for health. Sorghum superiority lies in the adaptability of archaeologists comprehensive drought tolerance of high production and greater resistance to pests and diseases (Nurmala, 1997).

Sorghum bicolor L. is one type of cereal crops that have great potential to be developed in Indonesia because the area has a wide adaptability. Sorghum plants tolerant to drought and waterlogging, can produce on marginal land and relatively resistant to gamma disorders / diseases. Grain sorghum can be used as food and feed industry raw materials and foods such as sugar, monosodium glutamate (MSG), amino acids, and the beverage industry. In other words, sorghum is a developer for industrial commodities vertically (Sirappa, 2003).

The area has the potential for the development of sorghum in Indonesia is very broad, encompassing dry climates or short rain season and infertile soil. Sorghum producing areas with traditional enterprise is java (Purwodadi, Pati, Demak, Wonogiri), 15 309 ha planting area, production of 17,350 tons with productivity 1:13 t / ha. Jogjakarta (Gunung Kidul, Kulon Progo) planting area of 1,813 ha, the production of 10 522 tonnes with a productivity 0:37 t / ha, East Java (Lamongan, Bojonegoro, Tuban, Probolinggo) planting area of 5,963 ha, the production of 10 522 tonnes with a productivity of 1.76 t / ha (Sirappa, 2003). Grain of sorghum amounted to 80.42% carbohydrate, 10:11% protein, 3.65% fat, 2.74% fiber and ash 2:24%.

Nutritional content of sorghum by cassava and taro are not much different from the carbohydrate to 35% cassava and taro 29%. The protein content of cassava taro 2.2% and 3%. The moisture content of 63% cassava and taro 57%. With high carbohydrate content, sorghum is also used as an alternative staple food and as a flour substitute some food products. Sorghum also contains glutenin and gliadin protein but less sorghum protein can form protein gluten than wheat flour (Suarni, 2004).

One of the obstacles in the processing of grain sorghum into food is high tanin content is about 3.67-10.66% Tannins can make sense of grain sorghum were poisoned. The content of tannins in sorghum also gives the effect of dark colors on the product so that the required reduction by milling tannin levels. Sorghum milling can reduce the levels of tannin and 75% (Suarni, 2004). The modified starch is starch treated with particular with the aim to produce better properties than previous properties or change some other properties. Starch can be broken down into units smaller by cutting ties glikosidiknya. One of the enzymes that can memtong the bonding is α-amylase enzyme (α-1,4 glukanhidrolase). The enzyme α-amylase can be obtained from various sources, for example, microorganisms such as Aspergillus oryzae and Bacillus subtillis (Koswara, 2009).

Fermentation is an activity of the microbes to use organic compounds or a source of carbon in order to obtain materials and energy metabolism in the joint production of gas. Carbon source in fermentation are carbohydrates, lipids, proteins and derivatives, while the role of microbes are bacteria, fungi and yeasts.

The fermentation process depends on the production of microorganisms, chemical and physical peribahan transfiguring, and the shape and flavor of the original food. The fermentation process also can improve the nutrition of the product as well as inhibit the growth of undesirable microorganisms. The success of a process of fermentation in order to obtain better products and quality compared to the original material, is closely

related to the treatment process. Factors that influence the process biokonveksi via fermentation is a type of microbe, inoculum concentration and fermentation time.

## 2. Metodhology

The materials used in the study was sorghum (Sorghum bicolor L monench) ± 4 months of age, subtillis Bacillus bacteria, molds and yeasts Aspergillus oryzae Sacharomyces cerevisiae obtained from laboratory Bandung Institute of Technology, and materials for analysis. The equipments used in the study was beakers, measuring cups, scales, tray, tunnel dryer, blender, vibratory screening, and equipment for chemical analysis.

The research was divided into two phases covering the preliminary study and the main study. The purpose of the preliminary study is to determine the best media concentration of sorghum to be used as a reference for the main study, the concentration is 200 grams, 250 grams, 300 grams. Determining the best media based on the analysis of the levels of dextrin (AOAC, 1995). Analysis of water content (AOAC, 1995), starch (AOAC, 1995), levels of dextrin (AOAC, 1995), protein content (AOAC, 1995), amylose content (IRRI, 2002) and amylopectin towards raw materials sorghum and sorghum flour without fermentation.

The draft response in this study include response to chemical, physical and organoleptic response. Chemical response carried out on primary research is the determination of water content by gravimetric methods (AOAC, 1995), Determination of dextrin Schrool Luff methods (AOAC, 1995), Determination of starch content with Luff Schrool methods (AOAC, 1995).

Physical response does is analysis of whiteness on the product selected by using the tool whiteness meter. Response organoleptic performed on primary research is covering the white color and aroma to the modified sorghum flour. Responses were performed on selected samples are spectrophotometric method Determination of amylose content (IRRI, 2002), Determination of amylopectin Determination of protein content (AOAC, 1995).

## 3. Results and Discussion

### Preliminary Research

Table 1 shows the results of the analysis of raw materials (grain sorghum) and unfermented sorghum flour there is little difference in water content. The water content of the raw materials (grain sorghum) amounted to 11.72%, while the moisture content of sorghum flour without fermentation at 11:57%. This difference is due to grain sorghum has undergone the process of drying and milling. The water content is an important parameter in flour products as they relate to product quality and product acceptability in the market. The lower the water content of a product the better the quality because low levels of water will reduce the possible growth of microorganisms that have an impact on increasing the durability of the product. According to Winarno (1997), water is an important component in food because of the water determines the appearance and texture.

Starch content of the raw materials (grain sorghum) amounted to 53.09%, while the starch content of sorghum flour without fermentation by 52.58%. There is little difference in starch content, resulting flouring process which causes loss of some amylose starch so that the starch content decreased slightly. Dextrin content of raw materials (grain sorghum) amounted to 2.66% while dextrin content of sorghum flour without fermentation by 2.72%. The protein content of raw materials (grain sorghum) amounted to 1.67%, while the protein content of sorghum flour without fermentation by 1.67%. There were no differences between the protein content of grain sorghum is intact with no fermented sorghum flour, flouring mechanical treatment did not reduce levels of protein.

Table 1. Analysis of Raw Material (Shorgum) dan Shorgum Fluor Without Fermentation

| Components | Komposisi | |
|---|---|---|
| | Raw material Shorgum (%) | Shorgum Flour Without Fermentation (%) |
| Water | 11.72 | 11.57 |
| Starch | 53.09 | 52.58 |
| Dextrin | 2.66 | 2.72 |
| Protein | 1.67 | 1.67 |
| Amilose | 27.33 | 26.46 |
| Amilopectine | 25.76 | 26.12 |

Table 2. Preliminary Results Making Koji (Sorghum Media Concentration Determination)

| Type of Microorganism and Medium Concentration (g) | Dextrin (%) |
|---|---|
| *Sacharomycecs cerevisiae* (200) | 6.37 |
| *Sacharomycecs cerevisiae* (250) | 8.15 |
| *Sacharomycecs cerevisiae* (300) | 8.82 |
| *Bacillis subtillis* (200) | 5.99 |
| *Bacillis subtillis* (250) | 8.87 |
| *Bacillis subtillis* (300) | 10.02 |
| *Aspergilus oryzae* (200) | 8.87 |
| *Aspergilus oryzae* (250) | 9.32 |
| *Aspergilus oryzae* (300) | 10.40 |

Amylose content in the raw materials (grain sorghum) amounted to 27.33% while the amylose content of sorghum flour without fermentation by 26.46%. For the amylopectin content of the raw materials (grain sorghum) amounted to 25.76% while the amylopectin content of sorghum flour without fermentation by 26.12%. There is little difference in the content of amylose and amylopectin in raw materials (grain sorghum) in sorghum without fermentation, as a comparison parameter.

The treatment used is to make koji with different concentrations of sorghum media with the help of microorganisms *Sacharomycecs cerevisiae*, *Bacillus subtillis* and *Aspergillus oryzae*, fermentation time of 48 hours. Parameter rising levels of dextrin produced a reference in determining the type and concentration of microorganisms best media.Starch can be broken down into units smaller by cutting ties glikosidanya. In the partial hydrolysis reaction, the starch molecules are broken into smaller known as dextrin. Dextrin is the result of the starch hydrolysis process before it is formed maltose. One enzyme that can cut the bonding is the enzyme α-amylase. Pure α-amylase enzyme can be obtained from various sources, for example from malt (barley), human saliva and pancreas. Can also be isolated from *Aspergillus oryzae* and *Bacillus subtillis*.

Speed enzyme reaction depends on the concentration of the substrate. However, at higher concentrations the reaction rate is no longer dependent on the concentration of the substrate. So at higher concentrations the reaction rate is not influenced anymore by the increase of the concentration. This shows that the enzyme seola if it had been 'saturated' with the substrate, meaning that it can no longer accommodate the substrate. (Poedjiadi and titin, 2009). Table 2 shows that the concentration of media 300 grams using microorganisms *Sacharomycecs cerevisiae*, *Bacillus subtillis* and *Aspergillus oryzae*, with 48-hour fermentation period greater yield dextrin.

**Primary Research**

The main research is a continuation of the preliminary research. The main study was conducted to determine the effect of concentration koji and fermentation time on the fermentation modified taro flour. Based on preliminary research showed that the microorganisms Sacharomyces cerevisiae, Bacillus subtillis and Aspergillus oryzae with media concentration of 300 grams. The draft response carried out on primary research was the response organoleptic and chemical response.

Response organoleptic done using hedonic quality test for color and aroma modified taro flour fermentation. chemical response includes the analysis of water content, levels of dextrin and starch content.

*a. Colour Response of Modified Shorgum Flour*

Color is a property of materials that ascribed spread-spectrum light, as well as the gloss properties of the material is influenced by light primarily reflected ray. The color quickly and easily make an impression but the most difficult in the given description and difficult way of measurement for a subjective judgment, that vision is crucial in the assessment of the commodity (Soekarto, 1985). The results of organoleptic test showed that there is no real difference between the length of fermentation, the koji concentration and the interaction between them so that no further testing is required.

Table 3. Effect of Fermentation Time to Flavour of Modified Sorghum Flour

| Fermentation Time (hour) | Average | Level |
|---|---|---|
| $a_1$ (24 h) | 2.85 | a |
| $a_2$ (36 h) | 3.45 | b |
| $a_3$ (48 h) | 3.90 | c |

Note: The average treatment followed by the same letter are not significantly different at the 5% significance level by LSD 0:05

*b. Flavour Response of Modified Shorgum Flour*

Aroma is defined as one that can be observed with the sense of smell. Ratings are aroma influenced by psychological and physiological factors that lead to different opinions (Winarno, 1997). Results of analysis of variance showed that there is no interaction between fermentation time and concentration of the aromas koji modified sorghum flour. But on the treatment effect on the length of fermentation aromas modified sorghum flour.

Based on Table 3 can be seen that the length of fermentation each treatment was significantly different with flavour of modified sorghum flour. Granules modified starch in sorghum flour will undergo hydrolysis and divided so as to produce simpler compounds and organic acids. Organic acids will terimbibisi in materials that can produce a distinctive aroma aroma cover original material. Moreover, the longer the fermentation process the increasing number of organic acids formation of the starch component, which decomposes proteins with increased activity of an enzyme produced (Poedjiadi and Titin, 2009). Flavour is one parameter in determining the quality of a food product. Distinctive aroma can be perceived by the senses of smell depends on materials and ingredients added to these foods. Thus aroma directly affects consumers' interest to try a food product. Flavour in foodstuffs can be generated by the components volatile, but volatile components may be lost during processing is too hot (Fellows, 1990).

*a. Water Content*

The water content in foodstuffs determined freshness and durability of materials. To prolong the durability of the most water in the material to be removed in an appropriate manner the type of material, such as by drying.

Table 4. Effect of interaction of Fermentation Time and Koji Concentration to Water Content Modified Sorghum Flour

| | Koji Concentration (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $b_1$ (2%) | | $b_2$ (4%) | | $b_3$ (6%) | | $b_4$ (8%) | | $b_5$ (10%) | |
| $a_1$ (24 h) | 8.73 C | a | 8.75 C | a | 8.96 C | a | 8.62 C | a | 8.59 C | a |
| $a_2$ (36 h) | 8.04 B | b | 7.91 B | b | 7.83 B | ab | 7.72 B | ab | 7.48 B | a |
| $a_3$ (48 h) | 6.92 A | c | 6.76 A | bc | 6.64 A | bc | 6.47 A | b | 5.65 A | a |

Note: The average treatment followed by a letter different showed significant difference according to a further test LSD at 5% level read horizontally lowercase and uppercase read vertically

Drying the flour has the objective to reduce the moisture content to a certain extent so that the growth of microorganisms and enzyme activity causes damage to the flour can be inhibited. Materials that have a high water content is usually more perishable than the material at low moisture content, the activities of microorganisms.The minimum limit moisture content where microbes can still grow is 14-15% (Fardiaz, 1992).

Results of statistical analysis using analysis of variance with a 95% confidence in attachment 1 indicates that the treatment is a long fermentation, the koji concentration and interaction between the two have real impact on water levels in sorghum modified. The presence of interactions requires you to check the influence of simple factors koji fermentation time and concentration. The results of variance analysis of the influence of the interaction between fermentation time and concentration koji can be seen in Table 4.

Table 4 shows that the interaction between the old and the concentration of koji fermentation gives a real difference to the moisture content of sorghum flour modified. Each treatment is a treatment in generating optimum moisture content.

Standard water content in the starchy flour is a maximum of 13-14.5%. The decline in water levels in sorghum modified is necessary given the water content can affect the storage process in sorghum. The decline in water levels lowest in a modified sorghum flour on a long fermentation koji 48 hours at a concentration of 10% with an average of 5.65%, this shows that the water content of the flour has met the quality requirements starchy. The water content of the flour is influenced by several things including the treatments that are experienced and old and product storage conditions.

*b. Starch Content*

Starch contained in grains is used as an energy supplier in the germination process or in the formation of the leaves on the plant. For humans the starch content in cereals used as a food to meet the needs of carbohydrates. Each type of plant has a biosynthetic process different in the formation of amylose chains and amilopektinya. From these different processes produced a variety of sizes, as well as the composition of amylose and amylopectin different produce types and properties of starch are different also in each source type of starch (Febuardi, 2012). Results of statistical analysis using analysis of variance with a 95% confidence in attachment 2 showed that treatment of long fermentation, the koji concentration and interaction between the two have real impact on the levels of modified starch in sorghum. The presence of interactions requires you to check the influence of simple factors koji fermentation time and concentration. The results of variance analysis of the influence of the interaction between fermentation time and concentration koji can be seen in Table 5.

Table 5 shows that the interaction between the old and the concentration of koji fermentation gives a real difference to the levels of starch in sorghum modified. The highest starch content is obtained from the fermentation 24 hours at a concentration of 2% of koji 44 750%, while the lowest starch content is obtained from the fermentation of 48 hours of treatment with koji concentration of 10% of 26 730%. Decreased levels of starch in sorghum modified compared to unfermented sorghum flour. this is because during the fermentation process occurs solving components of starch into simpler by microorganisms Sacharomycec cerevisiae, Bacillus subtillis, and Aspergillus oryzae that produces the enzyme amylase.

During the fermentation process takes place microbes will break down starch into simple sugars component so that the starch content. Starch is the substrate used by hydrolyzing enzyme amylase in starch into sugars more simple, amylase is an enzyme which is able to hydrolyze (break) binding α-1,4-glycosidic on amylose and amylopectin randomly liberate smaller units with edges has a cluster of non-reducing bebsas. The function of this enzyme is turning them into dextrin.
However, this has the disadvantage that the enzyme is not able to break the α-1,6-glycoside on pleh amylopectin hydrolysis because it results consisting of dextrin and other saccharides with a low molecular weight. Due to the influence of its activities, disjointed starch into dextrin with 6-10 units along the chain of glucose (Tranggono, 1990). Starch hydrolysis process can be seen in the following reaction:

$(C_6H_{10}O_5)_n + nH_2O \rightarrow (C_6H_{10}O_5)m.nH_2O + C_{12}H_{24}O_{12} + C_6H_{12}O_6$

*a. Dextrin Content*

Partial hydrolysis reaction, the starch molecules are broken into smaller known as dextrin. Dextrin is the degradation products in part a result of the activity of the enzyme, acid, and heating starch. The most complex degradation products intended as amilodekstrin or starch dissolved. Dextrin can be made easily from starch by means of heating. According to (Poedjiadi and Titin, 2009) dextrin is the result of the starch hydrolysis process before forming maltose. Results of statistical analysis using analysis of variance with a 95% confidence level contained in annex 3 shows that treatment of long fermentation, the koji concentration and interaction between the two have real impact on the levels of starch dextrin modified sorghum. The presence of interactions requires you to check the influence of simple factors koji fermentation time and concentration. The results of variance analysis of the influence of the interaction between fermentation time and concentration koji can be seen in Table 6.

Table 5. Effect of interaction of Fermentation Time and Koji Concentration to Wheat Starch Modified Sorghum

| Time Fermentation (hour) | Koji Konsentration | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $b_1$ (2%) | | $b_2$ (4%) | | $b_3$ (6%) | | $b_4$ (8%) | | $b_5$ (10%) | |
| $a_1$ (24 h) | 44.750 C | b | 44.655 C | b | 44.605 C | b | 43.325 C | a | 42.560 C | a |
| $a_2$ (36 h) | 40.475 B | d | 39.525 B | c | 38.715 B | c | 36.670 B | b | 35.605 B | a |
| $a_3$ (48 h) | 31.815 A | d | 30.055 A | c | 29.535 A | c | 28.350 A | b | 26.730 A | a |

Note: The average treatment followed by a letter Different showed significant difference according to a further test LSD at 5% level read horizontally lowercase and uppercase read vertically

Table 6. Effect of interaction of Fermentation Time and Koji Concentration Levels Against Dextrins Modified Sorghum Flour

| Fermentation Time (hour) | Koji Concentration (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $b_1$ (2%) | | $b_2$ (4%) | | $b_3$ (6%) | | $b_4$ (8%) | | $b_5$ (10%) | |
| $a_1$ (24 h) | 7.92 A | a | 8.75 A | b | 9.44 A | c | 9.89 A | d | 10.42 A | e |
| $a_2$ (36 h) | 8.62 B | a | 9.20 B | b | 9.77 A | c | 10.23 A | d | 10.58 A | e |
| $a_3$ (48 h ) | 9.39 C | a | 9.70 C | a | 10.48 B | b | 11.60 B | c | 13.02 B | d |

Note: The average treatment followed by a letter Different showed significant difference according to a further test LSD at 5% level lowercase and uppercase horizontal read-read vertically

Table 6 shows that the interaction between the old and the concentration of koji fermentation gives a real difference to the levels of starch in sorghum modified. The highest levels of dextrin obtained from fermentation time of 48 hours at a concentration of 10% by 13:02 koji%, while the lowest levels of dextrin obtained from the fermentation of a 24-hour long treatment with koji concentration of 2% at 7.92%.

Increased levels of amylase enzyme dextrin due to the many resulting effect on the process of hydrolysis of starch to produce dextrin-containing sugars reduction. The longer the fermentation time and the greater the concentration of koji meal will be generated from the hydrolysis of starch dextrin them.

One distinguishing between starch with dextrin is characteristic solubility in cold water. Pati has properties not dissolve in water because there are too many hydrogen bonds in the molecule. During the process of hydrolysis, long Ratai starch is converted into ratntai shorter, and produce simple sugars that are easier larutdalam cold water. This change causes dextrin soluble in cold water (Tjokroadikoesoemoe, 1986).

According to Winarno (1997) states that the starch can dihidrlolisis with amylase enzyme that can produce dextrin, maltose, maltotriosa, and isomaltosa. Dextrin resulting from hidrolisispati soluble in water because it can bind hydrophobic substances that can be used as a food additive to improve the texture of foodstuffs.

## Selected Product

The selected sample is obtained based on the results of chemical analysis was conducted on the analysis of water content, starch and dextrin levels, then can the selected products are modified sorghum flour sample dengen fermentation time of 48 hours (a3) with a concentration of 10% koji (b5¬). Selected product analysis modified sorghum flour whiteness of 64.60%, 1.67% protein content, amylose and amylopectin by 12.94% amounting to 13.79%.

## Conclusion

Fermentation time affect the water content, starch and dextrin content of sorghum flour modified. Concentration koji affect the water content, starch and dextrin content of sorghum flour modified. Interaction between koji fermentation time and concentration affect the water content, starch and dextrin content of sorghum flour modified. The selected products are modified sorghum flour sample with a concentration of 10% (b5) and fermentation time (a3) with a water content of 5.65%, 26.73% starch, dextrin content of 13:02%.

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## References

AOAC, (1995), *Official Methods of Analytical of The Association of Official Analytical Chemis,*. AOAC, Washington DC.

Fardiaz, S., (1992), Mikrobiologi Pangan 1, Edisi Pertama, P.T Gramedia Pustaka, Jakarta

Febuardi (2012), Teknologi Pati dan Gula, Hibah Penulisan Buku Ajar Bagi Tenaga Akademik, Universitas Hasanudin, Makasar.

Fellows, P., (1990), *Food Processing Technology*, First Edition, Elis Horword Limited, England.

Herry, R., Budhi, P dan Martanto M., (2010) Antioksidan dan Imunomodulator pada Serealia, Seminar Nasional Pendidikan Biologi, FKIP, Universitas Negri Sebelas Maret, Surakarta.

Koswara, S., (2009), Teknologi Modifikasi Pati, ebookpangan.com, Akses : 1 April 2015

Nurmala, T., (1997), Serealia, Cetakan Pertama, Rineka Cipta, Jakarta

Poedjiadi, A. dan F.M Titin Supriyanti, (2009), Dasar-dasar Biokimia, Edisi Revisi, UI-Press, Jakarta.

Richana N., Agus B. dan Ira M., (2010) Pembuatan Tepung Jagung Termodifikasi dan Pemanfaatannya untuk Roti, Balai Besar Litbang Pasca Panen.

Sirappa, M.P, (2003), Prospek Pengembangan Sorgum di Indonesia Sebagai Komoditas Alternatif untik Pangan, Pakan, dan Industri. Balai Pengkajian Teknologi Pertanian Sulawesi Selatan, Makasar.

Soekarto, (1985), Penilaian Organoleptik Untuk Industri Pangan dan Hasil Pertanian, Cetakan Pertama, Penerbit Bharata Karya Aksara, Jakarta.

Suarni, (2004), Pemanfaatan Tepung Sorgum untuk Produk Olahan, Jurnal Litbang Pertanian, Makasar.

Tranggono dan Sutardi, (1990), Biokimia dan Teknologi Pasca Panen, PAU Pangan dan Gizi, Universitas Gajah Mada, Yogyakarta

Tjokroadikoesoemoe, P.S, (1986), HFS dan Industri Ubi Kayu Lainnya, Edisi Pertama, Gramedia, Jakarta.

Winarno, F.G., (1997), Kimia Pangan dan Gizi, Cetakan Kelima, Gramedia Pustaka Utama, Jakarta.

# Evaluation Performance Of Some Hybrid Of Watermelon From Indonesian Tropical Fruit Research Institute

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## Abstract

Watermelon hybrid result from crossing by Indonesian Tropical Fruit Research Institute (ITFRI) has a high diversity. The objective of the research was to determine the performance of hybrid watermelon of ITFRI collection and to evaluate the superiority of each hybrid. The research has been conducted at Sumani Experimental Garden, ITFRI in April until July 2016. The material hybrid used consists of 19 cross combinations. The experiment was a descriptive study. The data was displayed in the form of average and pictures. Variables observed consisted of fruit weight, fruit length, fruit stalk length, thecircumference of fruit, rind thickness, rind color, flesh color, seed color and total soluble solids (TSS). Selection hybrid based on the character preferred by farmers as fruit weight ranged between 4-6 kg, red or yellow flesh color, sweet taste, and shelf life.The results showed that the hybrids were tested have diversity in size, fruit skin color, flesh color, skin thickness, and sweetness of the fruit. Have not been found hybrids yet that have three excellent characters, consisting of medium fruit weight, high in TSS, and rather a thick rind. A hybrid that has the advantage of a sweet taste and shelf life are BT1xSGP, BT3 x BT5, BT3 x BT6, BT4 x BT2, BT4 x BT4P, BT4 x BT6, and BT4xBT4P."

Keywords: watermelon, hybrids, TSS, performance

## 1. Introduction

Watermelon (Citrullus lanatus Thunb (Matsum) Nakai) is believed to originate from Africa and distributed to all regions of the world. This commercial plant has a water content more than 90% [1]; [2]. In addition, the content of lycopene in watermelon fruit pulp believed to reduce the risk of esophageal cancer [3]. Besides its flesh is rich with various kinds of vitamins, the rind also contains a polysaccharide compound these are widely used as anti-cancer, anti-coagulant, anti-virus and antioxidants [4].

Successfulness in watermelon breeding program in Indonesia is still very limited. Similar conditions also occur in Malaysia. The problem faced in the breeding program watermelon are the lack of genetic resources and environmental stresses such as high humidity, rain, and pests and diseases that cause crop failure. This problem causing are highly dependent on the commercial varieties released by foreign seed companies [5].

Hybrid breeding of cross-pollinated crops such as watermelon has several advantages when compared to open pollinated varieties, among others, in the increased value that leads to heterosis, and the stability of higher yields [6]. The watermelon breeding program initially aimed to acquire high-yielding varieties [7]. Furthermore, it also directed to get a variety that resistant to abiotic [8] and biotic stresses [9]. Besides, breeding watermelons began to lead to varieties with high nutrient content such varieties with containing high citrulline,

β-carotene, and lycopene. Breeding programs are also directed to assembly a variety that has related to flesh color and texture of the flesh of the fruit crisp [10], [11].

Indonesian Tropical Fruit Research Institute (ITFRI) until now has eight lines of watermelon that have been genetically stable. Eight lines consist of three numbers with the red flesh and five numbers to the yellow flesh color. Eight lines are expected to be used as a parent in getting the new varieties, both hybrids, and open pollinated varieties. This study is a stage of the evaluation of single cross hybrids resulting from a combination of cross different of inbred lines conducted by watermelon breeders of ITFRI. The study aims to determine the performance of hybrid watermelon ITFRI collection and to evaluate the superiority of each hybrid.

## 2. MaterialandMethods

The research was conducted at the Sumani Experimental Farm, ITFRI in April to July 2016. The material used were consists of 19 cross combinations, namely BT1 x BT3, BT1 x BT4, BT1 x SGP, BT3 x BT1, BT3 x BT5, BT3 x BT6, BT4 x BT2, BT4 x BT4P, BT4 x BT6, BT4 x BT4P, BT4P x BT4, BT4P x BT1, BT4P x BT5, BT5 x BT4, BT5 x BT1, BT5 x BT3, BT5 x BT4, BT6 x SGP, and SGP x BT6. The nineteenth result of crosses came from five lines with yellow flesh color and three lines in the red flesh. Each of these hybrids is planted in beds measuring 16 x 6 m, with total 30 plants per bed.

The variables observed have consisted of fruit weight, fruit length, fruit stalk length, a circumference of fruit, thick rind, fruit skin color, flesh color, seed color and total soluble solids (TSS). Selection hybrid based on the character preferred by farmers and consumers that have fruit weight ranged between 4-6 kg, red or yellow flesh color, sweet taste, and shelf life. The experiment is a descriptive trial. The data were displayed in the form of flats and images.

## 3. Result andDiscussion

The result shows that an accession number which has the highest fruit weight is BT4 x BT4P and BT5 x BT3 with the average fruit weight was 7.7 kg, while the lightest fruit was found in BT4P x BT1, i.e. 2.3 kg (Table 1). Based on discussions with farmers it can be concluded that consumers have different preferences on the weight of watermelons, i.e. farmers prefer with fruit weight produce 4-5 kg because of high production, while fruit consumers for household consumption prefers small-sized fruit with a maximum weight of 3 kg per fruit, as a direct fruit consumed one family without having saved if be left over.

Watermelon lines from ITFRI collection and cross-bred hybrids generally have around in shape, so the fruit has a rather short length, but the fruit has a circumference. The longest fruit came from crossbreeding lines BT4 x BT4P which are 22.7 cm and the shortest fruit came from crossing between BT3 x BT6, which is 15 cm. The largest fruit circumference is found in the hybrid result of crosses between lines BT4 x BT4P, which is 74.8 cm, and the smallest fruit circumference is found in the hybrid result from crosses between lines BT3 X BT6, which is 50 cm.

The thickest rind was found in the fruit that result of crosses between lines BT1 x SGP, which is 1.4 cm. The thinnest rind was found in the result of crosses between lines BT5 x BT3, and BT5 x BT1, which are 0.8 cm. The thickness of rind is usually associated with the shelf life of fruit. According to [12], thin-skinned fruit tends to have a short shelf life. Nevertheless, the fruit skin is too thick also did not satisfy requirements good quality fruit because it has a lower of edible portion.

Table 1. Characters are correlated with the hybrid watermelon production.

| | Accession | Fruit weight (kg) | Fruit length (cm) | Fruit circumference (cm) | Rind thickness (cm) | Color of rind | Flesh color | Seed color | TSS (°brix) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | BT1 x BT3 | 4.5 | 20 | 62.5 | 1.2 | light green striped | Red | Black | 9 |
| 2 | BT1 x BT4 | 4.2 | 21 | 61.5 | 1 | Light green striped | Red | Black | 9.8 |
| 3 | BT1xSGP | 5.6 | 23 | 67.3 | 1.4 | Plain dark green | Red | Black | 11.2 |
| 4 | BT3xBT1 | 5 | 21.4 | 66.3 | 1.2 | Plain dark green | Red | Black | 9.8 |
| 5 | BT3 x BT5 | 5.1 | 22 | 66.5 | 1.1 | Plain dark green | Red | Dark brown | 9.5 |
| 6 | BT3 x BT6 | 1.9 | 15 | 50 | 1.2 | Plain dark green | Red | Black | 10 |
| 7 | BT4 x BT2 | 5.3 | 21.9 | 66.8 | 1 | Plain light green | Yellow | Brown | 10.3 |
| 8 | BT4 x BT4P | 7.7 | 26 | 74.8 | 1.2 | Plain dark green | Yellow | Brown | 9 |
| 9 | BT4 x BT6 | 3.1 | 19 | 56 | 0.9 | Plain dark green | Yellow | Brown | 8.2 |
| 10 | BT4xBT4P | 6.8 | 25 | 73 | 1 | Plain dark green | Yellowish red | Black | 11.2 |
| 11 | BT4PxBT4 | 5.5 | 22.7 | 67.7 | 1.1 | Plain dark green | Yellowish red | Black | 9 |
| 12 | BT4P x BT1 | 2.3 | 17.5 | 51 | 1.1 | Plain light green | Yellow | Brown | 7.2 |
| 13 | BT4P x BT5 | 2.7 | 17 | 54 | 1 | Plain light green | Yellow | Brown | 8.2 |
| 14 | BT5 x BT4 | 5.4 | 21.7 | 66.1 | 1.1 | Light green striped | Yellow | Dark brown | 9.1 |
| 15 | BT5xBT1 | 4.9 | 21 | 66 | 0.8 | Plain dark green | Red | Black | 9.8 |
| 16 | BT5 x BT3 | 7.7 | 25 | 74 | 0.8 | Light green striped | Yellow | Black | 9.6 |
| 17 | BT5xBT4 | 4.1 | 20 | 62 | 1.1 | Plain dark green | Yellow | Black | 11.4 |
| 18 | BT6xSGP | 4.9 | 21.5 | 65 | 1.1 | Plain dark green | Red | Black | 11 |
| 19 | SGP x BT6 | 4.4 | 20.8 | 64.8 | 1.4 | Plain dark green | Red | Black | 10.2 |

Watermelon hybrid of ITFRI collection has three color variations rind namely light green striped, plain light green and plain dark green. According to [13] appearance of the fruit, skin is an important character in watermelon breeding, because of this character that was first seen by the consumer. Genetic studies are usually talking about the inheritance of rind color, rind stripe pattern, and fruit shape. Watermelon with light green striped rind color is commonly found on the market. Watermelon rind with plain dark green color, usually identical to the thick rind character, the texture of the rind is flexible, so unbreakable and shelf life. While the color plain light green allegedly formed by combining of two recessive characters during the formation of rind color. In a result of crossbreeding, light green rind color will usually be covered by a plain dark green rind and light green stripes. The result of crosses which have a plain light green color is BT4 x BT2 (Figure 1).

As we know, watermelon flesh color is usually red or yellow only. Red-yellow flesh color usually occurs when the female plants with red flesh color pollinated by the male flowers from yellow flesh color, or conversely. The fruit which has red-yellow flesh color is BT4 x BT4 P and BT4P x BT4 (Figure 2).

Total soluble solid usually associated with sweetness, even though the sweetness of the fruit is also influenced by the total acid. The fruit which has the highest TSS is BT4 x BT5 i.e. 11.4°brix, while the lowest TSS found in BT4P x BT1, i.e. 7.2 °brix.


Fig.1. Performance of ITFRI watermelon rind color (a) light green striped (b) plain dark green (c) plain light green.

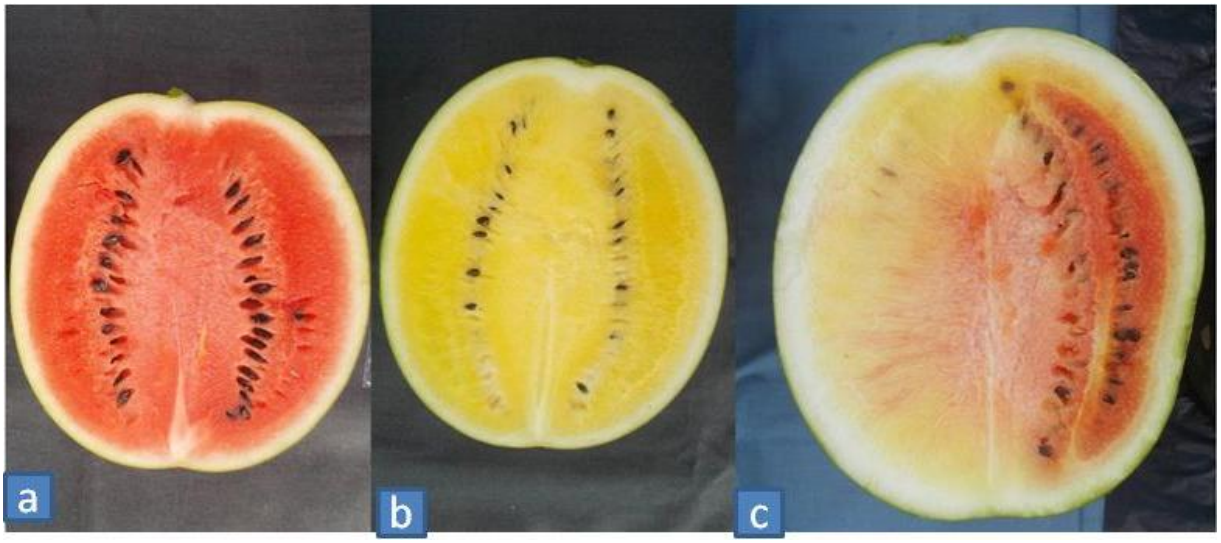


Fig.2. Performance of ITFRI watermelon flesh color

Table 2. Watermelon accessions have advantages over the fruit weight, TSS, and the thickness of the rind

| No. | Characters | Hybrids |
|---|---|---|
| 1. | Medium fruit weight ( 4-5 kgs) | BT1 x BT3, BT1 x BT4, BT3xBT1, BT5xBT1, BT5xBT4, BT6xSGP, SGP x BT6 |
| 2. | TSS (≥ 10 °brix) | **BT1xSGP**, **BT3 x BT5**, **BT3 x BT6**, **BT4 x BT2**, **BT4 x BT4P**, **BT4 x BT6**, **BT4xBT4P** |
| 3. | Rind thickness (1,1 - 1,3 cm) | BT1 x BT3, BT1 x BT4, **BT1xSGP,** BT3xBT1**, BT3 x BT5**, **BT3 x BT6**, **BT4 x BT2**, **BT4 x BT4P**, **BT4 x BT6**, **BT4xBT4P**, BT4PxBT4, BT4P x BT5, BT5 x BT4, BT5xBT1, BT5 x BT3, BT5xBT4, BT6xSGP, SGP x BT6 |

Based on the results of dialogue with local farmers that come from several partners in testing locations watermelon which has been implemented since 2009, concluded that watermelon fruit farmer and consumer demand from fruit that usually have a weight of 4-5 kg, sweetness (TSS ≥ 10 °brix), and a bit thick-rind so that is not easily broken during carriage (Table 2). Of the three excellent characters used as selection criteria, it turns out none of hybrid superior in all three of these criteria.

A hybrid which has the superiority of sweet taste and shelf life is BT1xSGP, BT3 x BT5, BT6 x BT3, BT4 x BT2, BT4 x BT4P, BT4 x BT6, and BT4xBT4P. Hybrid has the advantage of fruit weight 4-5 kg and sweetness not found.

## Conclusion

ITFRI have produced as many as 19 numbers of crossbred hybrid watermelon. The hybrid has the varying in size, rind color, flesh color, rind thickness and sweetness of the fruit flesh. It has not been found hybrids yet that have three excellent characters, which were fruit weight, TSS high, and rather a thick rind. Hybrid has the superiority of sweet taste and shelf life is BT1xSGP, BT3 x BT5, BT6 x BT3, BT4 x BT2, BT4 x BT4P, BT4 x BT6, and BT4xBT4P.

## References


[1] V. Kumar, A.M. Shirol, R. Mudge, N.Thammaih and P. Kumar, "Genotype x environmental interaction in watermelon (*Citrullus lanatus* Thunb.) genotypes for yield and quality traits", *Karnataka J. Agril. Sci*.vol 25, pp248-252. 2012.

[2] M. Prabhakar, S.S. Hebbar, and A.K. Nair, "Influence of various sources and levels of fertilizer applied through fertigation on hybrid watermelon grown in rabi-summer" *J. Hortl. Sci*,vol. 8, no.1, pp.60-64. 2013.

[3] A. Naz, M.S.Butt, M.T.Sultan, M.M.N Qayyum and R.S Niaz,"Watermelon lycopene and allied health claims", *EXCLI Journal*., vol.13, pp.650-666. 2014.

[4] M. Romdhane, A. Haddad, I. Ghazala, K.B. Jeddou, C.B. Helbert, S.E. Chaabouni, Optimization of polysaccharides extraction from watermelon rinds: Structure, functional and biological activities. *Food Chemistry*,vol. 216, pp. 355–364. 2016.

[5] M. Bahari, M.Y.Rafii, G.B. Saleh, and M.A. Latif," Combining ability analysis incomplete diallel cross of watermelon (Citrullus lanatus (Thumb.)Matsum. & Nakai)", *The ScientificWorld Journal.* pp 1-6. 2012.

[6] C.F.H.Longin, J. Muhleisen, H.P. Maurer, H. Zhang, M. Gowda and J.C. Reif, "Hybrid breeding in autogamous cereals", *Theor Appl Genet*,vol. 125, pp1087–1096. 2012.

[7] G. Gusmini and T.C. Wehner,"Foundations of yield improvement in watermelon", Crop Sci, vol 45, pp141–146. 2005.

[8] Wakindiki IIC, Kirambia RK, "Supplemental irrigation effects on yield of two watermelons (*Citruluslanatus*) cultivars under semi-arid climate in Kenya", *African Journal of Agricultural Research*, vol. 6, no.21, pp.4862-4870. 2011.

[9] A. Levi, J.A. Thies, W.P. Wechter, H.F. Harrison, A.M. Simmons, U.K. Reddy, and P. Nimmakayala, Z. Fei," High-frequency oligonucleotides: targeting active gene (HFO-TAG) markers revealed wide genetic diversity among *Citrullus spp*. accessions useful for enhancing disease or pest resistance in watermelon cultivars", *Genet Resour Crop Evol.*,pp 1-14. 2012.

[10] Y. Park and S. Choi," Watermelon production and breeding in South Korea", *Israel J. Plant Sci*.,vol. 60, pp. 415-423. 2012.

[11] H. Kim, D. Han, J. Kang, Y. Choi, A. Levi, G.P. Lee, and Y. Park, "Sequence characterized amplified polymorphism markers for selecting rind stripe pattern in watermelon (Citrullus lanatus L.)", *Hort. Environ. Biotechnol.*, vol.56, no.3, pp341-349. 2015.

[12] Kuswandi, Sobir, dan W.B. Suwarno, "Keragaman genetik plasma nutfah rambutan di Indonesia berdasarkan karakter morfologi*", J.Hort*, vol. 24, no.4, pp 289-298. 2014.

[13] S.W. Park, K.T. Kim, S.C Kang, and H.B. Yang, *Rapid and practical molecular marker development for rind traits in watermelon*, Korean Society for Horticultural Science and Springer. 2016.

# The Using of Thidiazuron to Induce the Mangoesteen Shoot (G*arcinia mangostana* L.) by Direct Organogenesis

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## Abstract

Mangosteen until now generally propagated by seeds but it has some obstacles and barriers. The *in vitro* culture is the most appropriate way to multiply mangosteen seeds rapidly, mass, and uniform. The research aimed to determine the thidiazuron ability to induce the mangosteen shoots by direct organogenesis. The experiment was conducted at the Tissue Culture Laboratory, Indonesian Tropical Fruit Research Institute, from August to December 2012. Explants used were mature mangosteen seeds. The research used five kinds of PGR composition , ie : 1) WPM + 5 mg/l BAP (control), 2) WPM + 0,05 mg/l TDZ + 500 mg/l PVP, 3) WPM + 0,1 mg/l TDZ + 500 mg/l PVP, 4) WPM + 0,5 mg/l TDZ + 500 mg/l PVP, and 5) WPM + 1 mg/l TDZ + 500 mg/l PVP. The cultures were incubated in the room without light for 16 weeks with a temperature of 25 - 28$^{o}$C. The results showed that using of 0,05 – 1 mg/l thidiazuron was not effective to induce the shoot formation of mangosteen by direct organogenesis. The using of WPM basic media enriched with 5 mg/l BAP was the best treatment to induce the shoots from the mangosteen seeds by *in vitro* culture.

Keyword: Mangosteen, Induction, Shoot, Thidiazuron

## 1. Introduction

Mangosteen (*Garcinia mangostana* L.) is known as “Queen of tropical fruit” because it has unique taste, lovely shape and smooth texture fruit [1]. This fruits has been use for several medicinal properties, such as preventing obesity, dietary supplement, antibacterial, antifungal, antiinflammatory, antioxidant, antiplasmodial, cytotoxic, and potential cancer chemopreventive activities [2,3].

Mangosteen until now generally propagated by seeds. Propagation through seeds has some obstacles and barriers. These plants categorize slow-growing plant, seen from the growth of young leaves only once a year. Generally, mangosteen plants have been fruiting after the age of 15 years [4]. Seeds are only available on the fruiting season (1-2 times a year), each fruit only produces 1-2 seeds that are large and worthy to be used as seed.

The solving of that problem is through *in vitro* culture, *in vitro* culture is the most appropriate way to multiply mangosteen seeds rapidly, mass, and uniform [5]. The propagation methods through in-vitro culture can produce a clonal seedling in large numbers and relatively short time. Until now, it has been widely reported the successful of in-vitro culture of mangosteen. The research results in Singapore showed that the best shoot multiplication was obtained from WPM + BA 5 mg/l [6]. The using of MS media with the addition 9 mg/l BAP also produced the best shoot multiplication [7]. The using of shoot bud as explants source, with the addition 0.5 ppm BA + 1 ppm GA3 produced 42% number of explants grew [8].

Beside BA or kinetin, the using of thidiazuron (TDZ) may also increase the ability of shoot multiplication. TDZ has the same role in promoting *in vitro* via organogenesis or embryogenesis [9]. TDZ can induce the formation of adventitious shoots and proliferation of axillary shoots [10].

TDZ is an organic compound that is widely used in the in-vitro propagation because its activity resembles cytokine [11,12]. It has been used for *Ephedra gerardiana* [13], *Arachis paraguariensis* [9], *Cassia alata* L. [14], *Arachis hypogaea* L. [15], *Silybum marianum* [16], *Hedychium coronarium* [17], *Brassica rapa var. Turnip* [18], and *Aconitum balfourii* Stapf. [19]. TDZ used in micropropagation of several woody species and it has ability to stimulate shoot proliferation [13].

The purpose of this study was to determine the thiadizuron ability to induce the mangosteen shoots through direct organogenesis.

## 2. Materials and Methods

The experiment was conducted at the Tissue Culture Laboratory, Indonesian Tropical Fruit Research Institute, from August to December 2012. Explants used were mature mangosteen seeds. Explants husk cleaned, then sterilized using 70% alcohol for 5 minutes and 10-20% clorox for 10 minutes. Explants is cut into 3 pieces and then dipped in $HgCl_2$ 0.01 mg/l for 5-10 seconds. After that, the explants were rinsed with sterile distilled water three times then explants were cultured in Woody Plant Medium (WPM) that contain 30 g/l sucrose and 2,5 g/l phytagel. This experiment used 5 different plant growth regulator (PGR) compositions as follows:

1. 5 mg/l BAP (control)
2. 0,05 mg/l TDZ + 500 mg/l PVP
3. 0,1 mg/l TDZ + 500 mg/l PVP
4. 0,5 mg/l TDZ + 500 mg/l PVP
5. 1 mg/l TDZ + 500 mg/l PVP

The research design was Completely Randomize Design with 5 treatments, 3 replications and 3 samples (bottle). The cultures were incubated in a room without light for 16 weeks with temperature of 25-28$^{o}$C. Subcultures performed at intervals of 8 weeks to the same media. Variables that are measured at 8$^{th}$ week are shoot formation (%) and shoot number/bottle. Variables that are measured at 16$^{th}$ week are shoots formation (%), shoot number/explants, and the leaves number/shoots.

Data were analyzed statistically by F test and if the results F count larger than F table value of 5%, followed by HSD at the 5% significance level.

## 3. Results and Discussions

The result showed that the explants percentage to form the highest shoot (88,9%) contained on the using of WPM basal media enriched with 5 mg/l BAP. This was followed by the using of basal media WPM + 0.05 mg/l TDZ + 500 mg/l PVP (44,4%), WPM + 0.5 mg/l TDZ + 500 mg/l PVP (33,3%), WPM + 1 mg/l TDZ + 500 mg/l PVP (33,3%) and WPM + 0.1 mg/l TDZ + 500 mg/l PVP (11,1%). The using of TDZ with the concentration 0.05 mg/l - 1 mg/l TDZ is less effective to form the shoot because the shoot formation (%) is low (Table 1).

Most of shoots number in 8 weeks after initiation also obtained on the using of WPM basal media enriched with 5 mg/l BAP. Followed by using of WPM basic media enriched with0.05 mg/l - 1 mg/l TDZ + 500 mg/l PVP that only produce shoots 0,1 to 0,7 shoots/bottle. The efficiency of shoot regeneration was a quantitative characteristic that often varies between plant species, subspecies, varieties, cultivars, or ecotypes [9].

On the five treatments of PGR, all treatments produced shoots (Table 2). The highest shoot formation (100%) produced on the using of WPM basal media enriched with 5 mg/l BAP. Followed by the using of WPM basal media enriched with 0.05 mg/l - 1 mg/l TDZ + 500 mg/l PVP is only able to form

33,3% - 55,5% shoots. Basal media WPM combine with 0,1 mg/l TDZ + 500 mg/l PVP in mangosteen, only capable to produce 33,3% explants to shoot formation, this treatment significantly different with treatment WPM + 5 mg/l BAP.

Shoots that are produced in this study ranged from 0.3 to 18,8 shoots/explants. The highest shoots number (18,8 shoots) are produced on the using of WPM basal media enriched with 5 mg/l BAP while the consumption of WPM basal media enriched with 0.05 mg/l - 1 mg/l TDZ + 500 mg/l PVP is only able to form shoots from 0.3 to 1,1 shoots/explants. WPM basal media enriched with 5 mg/l BAP significantly different in shoot number with the other treatment.

Leaves number/shoots that are produced ranging from 0.60 - 3.60 leaves/shoot (Table 2). The most of leaves number/shoots (3,60) there are at the WPM media enriched with 5 mg/l BAP, this treatments was significantly different from the other treatments.

TDZ potentially stimulate the regeneration frequency in groundnut (*Arachis hipogaea*) in vitro, and stimulate the formation of adventitious shoot on some plants [20]. In some plant species, TDZ is quite influential in the shoot formation but at the research of *in vitro* culture of mangosteen by direct organogenesis, this is seen that the using of 0,05 mg/l – 1 mg/l TDZ is ineffective to induce the mangosteen shoots. The concentration of TDZ that used in this research was not effective to induce number of shoot in mangosteen.

TDZ have different effect for *in vitro* propagation, depending on plant species, variety, and concentration of TDZ. The research in *Arachis paraguariensis* using combination of TDZ and BAP have different effect depending on the concentration [9]. In one case, the increase concentration of TDZ followed by the increasing of shoot number but in different plant species, it has reverse effect.

Table 1. The effect of treatment of PGR on the shoot formation and shoot number on mangosteen *in vitro* culture 8 weeks after initiation (WAI).

| No | Media | Shoot formation (%) | Shoot number/explants |
|---|---|---|---|
| 1 | WPM + 5 mg/l BAP | 88,9 a | 10,2 a |
| 2 | WPM + 0,05 mg/l TDZ + 500 mg/l PVP | 44,4 a | 0.7 a |
| 3 | WPM + 0,1 mg/l TDZ + 500 mg/l PVP | 11,1 a | 0,1 a |
| 4 | WPM + 0,5 mg/l TDZ + 500 mg/l PVP | 33,3 a | 0,3 a |
| 5 | WPM + 1 mg/l TDZ + 500 mg/l PVP | 33,3 a | 0,3 a |

Table 2. The effect of treatment of PGR on the shoot formation, shoots number and leaves number at mangosteen *in vitro* culture 16 weeks after initiation

| No | Media | Shoot formation (%) | Shoot number/explants | Leaves number/shoot |
|---|---|---|---|---|
| 1 | WPM + 5 mg/l BAP | 100 a | 18,8 a | 3,5 a |
| 2 | WPM + 0,05 mg/l TDZ + 500 mg/l PVP | 44,4 ab | 0,7 b | 2,3 b |
| 3 | WPM + 0,1 mg/l TDZ + 500 mg/l PVP | 33,3 b | 0,3 b | 2,0 b |
| 4 | WPM + 0,5 mg/l TDZ + 500 mg/l PVP | 44,4 ab | 0.4 b | 2,3 b |
| 5 | WPM + 1 mg/l TDZ + 500 mg/l PVP | 55,5 ab | 1,1 b | 2,0 b |

In peanuts, the increase concentration of TDZ increased number of shoots, shoot primordia per explant and rate of shoot formation[15]. The use of TDZ in *Hedychium coronarium* in lower concentration already raise *in vitro* plantlets [17]. In *Silybum marianum*, the increase of TDZ concentration beyond the optimal level will decrease shoot organogenesis parameters [16].

In *Ephedra gerardiana*, TDZ in higher concentration use for callusing (indirect organogenesis) and in lower concentration use for direct organogenesis [13]. Other research reported that TDZ produced lower shoot number even failed to obtain shoot in organogenesis, when combine with NAA [16,21].

Number of mangosteen shoots that produced in WPM + 5 mg/l BAP is higher than the other study [22]. The highest of shoots number that be produced in this study is 18.8 shoots/seeds (used basal media WPM) and at the other research (used basal media MS), produced 2.7 shoots/seeds [22]. The high of shoots number that produced, beside affected by plant growth regulator that be used, is also influenced by the type of basal media used [23].

## Conclusion

The using of 0,05 – 1 mg/l thidiazuron is less effective to induce the shoot formation of mangosteen through direct organogenesis because it is only capable to form the shoot from 0.30 to 1.00 shoots/seed. In this study the using of WPM basal media enriched with 5 mg/l BAP is the best treatment to induce the shoots of mangosteen seeds through in-vitro culture.

## References


[1] D. Fairchild, "The Mangosteen". J. Hered., vol. 6(8), pp 339-347, 1915.

[2] A.S. Abuzaid, E.Y. Sukandar, N.F. Kurniati and I.K. Adnyana, "Preventive Effect on Obesity of Mangosteen (*Garcinia mangostana* L.) Pericarp Ethanolic Extract by Reduction of Fatty Acid Synthase Level in Monosodium Glutamate and High-Calorie Diet-Induced Male Wistar Rats". Asian Journal of Pharmaceutical and Clinical Research., vol. 9(3), pp 257-260, 2016.

[3] Y.W. Chin and A.D. Konghorn, "Structural Characterization, Biological Effects, and Synthetic Studies on Xanhones from Mangosteen (*Garcinia mangostana*), a Popular Botanical Dietary Supplement". Mini Review in Organic Chemestry., vol. 5, pp 355-364, 2008.

[4] M. Winarno, H.H. Sunarjono, Ismijati, dan S. Kusumo, "*Teknik Perbanyakan Cepat Buah-Buahan Tropik*". Jakarta : Pusat Penelitian dan Pengembangan Hortikultura, 1990.

[5] C.P. Victorio, C.L.S. Lage, and A. Sato. "Tissue Culture Techniques in the Proliferation of Shoots and Roots of *Calendula officinalis*". Revista Ciencia Agronomica., vol. 43, pp 539-545. 2012.

[6] H.K.L. Goh, A.N. Rao, and C.S Loh, "Direct Shoot Bud Formation from Leaf Explants of Seedlings and Mature Mangosteen (*G. mangostana* L.) Trees". Plant Sci., vol. 68, pp 113-121, 1990.

[7] M.N. Normah, A.B. Noor-Azza, and R. Aliudin, "Factors Affecting *In Vitro* Proliferation and *Ex Vitro* Establishment of Mangosteen". Plant Cell Tissue Organ Cult., vol. 43(3), pp 291-294, 1995.

[8] R. Triatminingsih, E. Nazir, dan M. Winarno. "Mikropropagasi *In Vitro* dari Tunas Pucuk Manggis dan Kotiledon terhadap Keberhasilan Regenerasi Tunas". Jurnal Hortikultura., vol. 5(2), pp 28-36, 1993.

[9] O. Aina, K. Quesenberry, and M. Gallo, " Thidiazuron-Induced Tissue Culture Regeneration from Quartered-Seed Explants *of Avachis Pamguariensis*". Crop Sci., vol 52, pp 1076-1083. 2012.

[10] C.Y. Lu, "The Use of Thidiazuron in Tissue Culture". In Vitro Cell Dev. Biol., vol. 29, pp 92-96, l993.

[11] R.L.M. Pierik, "*In Vitro Culture of Higher Plants*". London : Martinus Nijhoff Publisher. l987

[12] S. Singha, and S.K. Bathia, "Shoot Proliferation of Pear Culture on Medium Containing Thidiazuron and Benzyl Aminopurine". Hort. Sci., vol. 23, pp 803-806, l988.

[13] V. Sharma, S.K. Gupta and M. Dhiman, "Regeneration of Plants from Nodal and Internodal Segment Cultures of *Ephedra gerardiana* using Thidiazuron". Plant Tissue Cult. & Biotech., vol 22(2), pp 153-161, December 2012.

[14] M.R. Ahmed, N. Ahmad, M. Anis and I.M. Aref, "Efficient Plantlet Regeneration from Cotyledonary Node Cultures of *Cassia alata* L. (Ringworm Bush): Stimulatory Effect of

Thidiazuron". Journal of Horticultural Science & Biotechnology., vol. 88(2), pp 141–146, 2013.

[15] K. Matand, N. Wu, H. Wu, E. Tucker, and K. Love, "More Improved Peanut (*Arachis hypogaea* L.) Protocol for Direct Shoot Organogenesis in Mature Dry-Cotyledonary and Root Tissues". Journal of Biotech Research., vol. 5, pp 24-34, 2013.

[16] M.A. Khan, B.H. Abbasi, and Z.K. Shinwari, "Thidiazuron Enhanced Regeneration and Silymarin Content in *Silybum marianum* L.". Pak.J.Bot., vol 46(1), pp 185-190, 2014.

[17] M. Verma and Y.K. Bansal, "Effect of a Potent Cytokinin Thidiazuron (TDZ) on *In Vitro* Regeneration of *Hedychium coronarium* J. Koenig – A Valuable Medicinal Plant. International Journal of Recent Biotechnology., vol. 2(1), pp 38-44, 2014.

[18] K.U. Kakar, Z. Nawaz, B.H. Abbasi, and M. Ali. Thidiazuron Induced Plant Regeneration in *Brassica rapa* Var *Turnip* Via Seed Derived Calli Induction and Radical Scavenging Activity". Sarhad J.Agric., vol. 30(2). Pp 232-240, 2014.

[19] M. Gondval, P. Chaturvedi, and A.K. Gaur, "Thidiazuron-Induce High Frequency Establismnet of Callus Cultures and Plantlet Regeneration in *Aconitum balfourii* Stapf.: en Endagered Medicinal Herbs of North-West Himalayas,". Indian Journal of Biotechnology., vol. 15, pp 251-255, April 2016.

[20] C.A. Huetterman and J.E. Preece. "Thidiazuron: a Potent Cytokinin for Woody Plant Tissue Culture". Plant Cell Tissue Organ Cult., vol. 33, pp 105–119, 1993.

[21] G.M. Osman, E.A. Elhadi and M.M. Khalafalla, "Callus Formation and Organogenesis of Tomato (*Lycopersicon esculentum* Mill, C.V. Omdurman) Induced by Thidiazuron". Afr. J. Biotechnol., vol. 9(28), pp 4407-4413, 2010.

[22] I. Rostika, N. Sunarlim., dan I. Mariska, "Micropropagation of Mangosteen". Indonesian Journal of Agriculture., vol. 1(1), pp 28-33, 2008.

[23] Y.Z Joni and R. Triatminingsih, "Shoot Induction and Multiplication of Mangosteen (*Garcinia mangostana* L.) through *in vitro* culture". Proc. of national conference technology innovation of national horticulture, Lembang, West Java. July 5st, 2012.

# Healing Quality Of Femoral Fractures In Ovariectomized Rats With Therapy Of *Cissus Quadrangularis* Extract Shown by The Expression Of Type I Collagen

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## Abstract

In bone, the main component of the organic matrix is typed I collagen and the crystal phase consists mainly of dahllite (carbonated hydroxyapatite). The organic material gives bone its flexibility, while the inorganic material gives bone its resilience. This study aims to determine the quality of healing fractures in osteoporotic rat ovariectomized with extract *Cissus quadrangularis*, by measuring the expression of type I collagen, because it contributes to flexibility and tensile strength in theboneso that it can function properly toward a normal bone. This study used forty female rats weighing 200–250 g. The rats were randomly assigned to the sham-operated (SX, n = 10) or ovariectomized (n = 30) groups. The treatment started to be given the next day after osteotomy for six weeks. Rats in P0(NOV) and P1(OV) group received 1.5 ml 0.5% NaCMC per-oral as placebo. P2 group was treated using Raloxifene 5,4 mg/kg of body weight and P3 group was treated using *Cissus quadrangularis*extract 750 mg/kg of body weight. Observation the expression of type I collagen in this study were performed twice. The first observation was done two weeks after osteotomy and the second observation was done six weeks after osteotomy. Based on the statistical test of the expression type I collagen at the 2nd week, the highest score belongs to the P0 group (NOV) that have significant difference ($p<0.05$) with other groups of treatments, while the lowest score belongs to group P1 (OV) that have significant difference ($p<0.05$) with other groups of treatments. There is no significant difference at the P2 and P3 groups ($p>0.05$) At the $6^{th}$-week observation, expression type I collagen of P0, P2, and P3 was significantly higher ($p < 0.05$) than expression type I collagen of P1. The concluded that *Cissus quadrangularis* extract can increase the expression type I collagen on femoral fractures in ovariectomized rats so that they were has a large impact on the mechanical properties of bone and stronger resistance to mechanical loading with healing over time.

*Keywords:* type I collagen, osteoporosis, fracture, ovariectomized, *Cissus quadrangularis*

## 1. Introduction

Fractures are common complications of osteoporosis in older peopleand also animals that do ovariectomize, but there have been few studies of the healing process of fractures in osteoporotic bones. The healing of a fracture involves a complex series of cellularevents and these may be further complicated by osteoporosis [1].Poor bone quality in patients with osteoporosis presents the surgeon with difficult treatment decisions. Manipulation of both the local fracture environment in terms of theapplication ofgrowth factors, scaffolds, and mesenchymal

cells, and systemic administration of agents promoting bone formation and bone strength has been considered as a treatment option from which promising results have recently been reported. Surprisingly,less importance has been given to investigating fracture healing in osteoporosis [2].

It is expected that anabolic agents used to treat osteoporosis would have a beneficial effect on fracture healing. However, most patients who need treatment for osteoporosis will currently receive anticatabolic agents, and it is important to know whether this may have any disadvantage for the healing of incident fractures. The development of agents that possess osteogenic activity will enhance the above process. In the perspective of utilization of biologically active natural osteogenic agents, the plant species possessing medicinal properties that are being used in the ancient medicines are gaining much scrutiny of researchers. The osteogenic cells derived from mesenchymal or stromal stem cells uniquely elaborate the matrix of bone. These cellsdifferentiate into mature osteoblasts, which then actively synthesize and mineralize bone matrix[3].

*Cissus quadrangularis* is one such medicinal plant possessing osteogenic activity and is attaining increasing interest of their potential therapeutic agent to enhancing bone healing. This plant has been customarily used in the bone fracture healing and also known as abonesetter for its ability to join bones[4]. Phytochemical analyses of CQ have revealed highcontents of ascorbic acid, carotene, anabolic steroidalsubstances, and calcium [5]. The stem contains two asymmetric tetracyclic triterpenoids and two steroidal principles. The presence of β-sitosterol, δ-amyrin, δ-amyrone, and flavonoids (quercetin) has also bee reported. All of these components have potentially different metabolic and physiologic effects[6].

Sufficient stabilization of fractures in the weight-bearing extremities is the primary goal of treatment.The mechanical properties of bone reflect the inherentmaterial properties of its constituents and the way in which they are arranged and interact. In all connective tissues, collagen has mechanical functions, providing elasticity and structure for the component tissues. In bone, a large body of evidence indicates that type I collagen molecules are involved in mechanical properties of bone. Several studies indicate that collagen plays a substantial role in its toughness (capacity to absorb energy), while the mineral content is mainly involved in determining bone stiffness[7].

This study aims to determine the quality of healing fractures in osteoporotic rat ovariectomized with extract *Cissus quadrangularis*, by measuring the expression of type I collagen, because it contributes to flexibility and tensile strength in boneso that it can function properly toward a normal bone

## 2. MaterialandMethods

### 2.1. Animals

Forty female rats (*Rattus Norvegicus*) weighing 200–250 g were purchased from the Laboratory Animal Resource Unit, Faculty of Veterinary Medicine, Universitas Airlangga. The rats were housed individually in clean cages at room temperature with a normal 12-hour light-dark cycle and free access to water and food. The rats were acclimatized for ten days before they were randomly assigned to the sham-operated (SX, n = 10) or ovariectomized (n = 30) groups. Following a previously described protocol, the rats in the SX group underwent a sham operation, whereas the rats in the ovariectomized group underwent a bilateral ovariectomy at the beginning of the study [8]. This study was approved by the Animal Ethics Committee of Faculty of Veterinary Medicine Universitas Airlangga.

### 2.2. Research Procedure

#### 2.2.1 Preparation of Extract

The fleshy stems and leaves will be washed and cut into small pieces, air-dried and crushed into a powder. The powder was

extracted with 95% ethanol using a Maceration extraction.

### 2.2.2. Osteotomy Procedure

Osteoporosis was allowed to develop in the animals for eight weeks after the ovariectomy. A pilot study was carried out prior to the main study to confirm the induction of osteoporosis based on SEM analysis. Following a previously described protocol, the right femur of all rats underwent a closed fracture at mid-diaphysis with the installation of anintramedullary pin. The length of the pin that would be needed was measured with Vernier caliper, based on the length of the femur from radiograph result to determine the length needed. The osteotomy was conducted under anesthetized using acombination of Xylazine and ketamine (1:1) at an intramuscular dose of 0.1 ml/100 g (Troy Laboratories, Australia)[9].

To approach the diaphysis of thefemur, atransverse incisionwasmade alongthelaterallinecranialofmajortrochantertot he patella bone.The bonestem wasseparatedfrom the surroundingmuscles. The middle of thediaphysis ofthe femurwas cut by using thebonesaw.Intramedullarypin, a Kirschner wire (K-wire, 1.0 mm) will be mountedin the following way. The pinto be insertedfirstmeasuredlength isadjustedto the length ofthe femurbone. The pinis insertedintothe medullarycanalof the femurthroughthe end of thebroken bone fragmentsat thedistalanddrivenuntilthe pincannot bemovedagain. The partof theproximalbonefragment is takenand the top end of the pin is pushed into the proximal bone fragment of the medullary canal until the bone connected perfectly (fracture lines straight and the bone ends do not overlap), then closed the operation wound.

X-ray images were immediately obtained post-fracture to confirm both the intramedullary placement of the K-wire and the fracture. To prevent infection, 5% Enrofloxacin was given at a dose of 10 mg/kg I.M. (Bayer, Thailand) daily for 2 days with the daily dressing.

### 2.2.3 Treatment

The treatment started to be given the next day after osteotomy for six weeks. Rats in P0 (NOV + osteotomy) and P1 (OV + osteotomy) group received 1.5 ml 0.5% NaCMC per-oral as placebo. P2 (OV + osteotomy) group was treated using Raloxifene 5, 4 mg/kg of body weight and P3 (OV + osteotomy) group was treated using *Cissus quadrangularis*extract 750 mg/kg of body weight. Raloxifene and *Cissus quadrangularis*extract doses were based on previous research of Potu*et al.,* [10] which studied the effect of *Cissus quadrangularis*to osteoporotic rats.

### 2.3 Samples Observation andExamination

Treatment was given daily for six weeks via oral gavage immediately after fracture of the right femur. Examination of expression of type I collagen in this study performed twice. The first examination was done two weeks after osteotomy and the second examination was done six weeks after osteotomy.

Examination results in six weeks are in addition to being compared among treatment groups with the two-week post osteotomy. Five rats were taken from each treatment groups and anesthetized. After euthanasia, the right femur was dissected, and the K-wires were removed, and the tissue blocks of the fracture areas were fixed in 10% formalin foe 24 h, decalcified in EDTA and embedded inparaffin. The block was then cut into 4-µm slices along the sagittal plane passing through the longitudinal axis of the femur. For immunohistochemistry, the sections were incubated in 3% H2 O2 for 15 min to inhibit endogenous peroxidase activity after undergoing deparaffinization and rehydration. Following washing with distilled water, the sections were incubated in TBS with polyclonal antibodies against type I collagen diluted at 1:200 for 30 min. After 10-min

washing with TBS, the sections were incubated with Envision TM for another 30 min. After washing with TBS for 10 min, the sections were incubated with diaminobenzidine (DAB) as a substrate and counterstained. The results were evaluated by microscopy. All samples were classified according to the percentage of type I collagen positive cells (brown color reaction).

### 2.4. Statistical analysis

The statistical analyses were performed using the SPSSstatistical package version 20.0. Normally distributed data were presented as mean plus minus standard deviation (SD). The normally distributed variables were analyzed using an ANOVA model followed by LSD posthoc test.

## 3. Result andDiscussion

### Expression Of Type I Collagen

The examination of type I collagen expression was done in the 2nd week and the 6th week after osteotomy through the immunohistochemistry. This examination was intended to assess type I collagen expression at the beginning and the end of the fracture healing process. Table 1 shows statistical analysis of type I collagen expression at the 2nd and the 6th week after osteotomy.

Based on the statistical test of the type I collagen expression at the 2nd week, the highest score belongs to the P0 group (NOV) that have significant difference ($p<0.05$) with other groups of treatments, while the lowest score belongs to group P1 (OV) that have significant difference ($p<0.05$) with other groups of treatments. There is no significant difference at the P2 and P3 groups ($p>0.05$).

From the expression of type I collagen at the 6th weeks, the same pattern occurs in the 2nd week, the highest expression belongs to the P0 groups (NOV), but these results were no significant different ($P> 0.05$) with group P3 namely the rat ovariectomy therapy Cissus quadrangularis extract (CQ), but P0 significantly different ($P <0.05$) with a group P2 (ovariectomy with raloxifene therapy ) and P1 (ovariectomy without therapy). The lowest belongs to the P1 (OV) that have significant different with the other groups.

The comparison type I collagen expression between the 2nd and the 6th weeks at almost all of the groups shows significant different ($p<0.05$), only P1 groups shows no significant difference between the 2nd and the 6th groups ($p>0.05$). Based on the graphic of type I collagen expression, the 6th week is higher than the 2nd week (Figure 1.). This result shows that the expression of type I collagen is slightly higher at the remodeling phase (6th week). There is no significant increase in the expression of type I collagen during the process of fracture healing in group P1, so it is likely to cause delayed union even non-union fracture healing in ovariectomized rats as animal models of osteoporosis.

**Table 1:** Mean rate and deviation standard expression of the TGFβ-1 on the fracture healing process of rats *(Rattus norvegicus)* on each group of treatments.

| No. | Groups of treatment | Ekspresi Kolagen tipe-1 (2nd weeks) | Ekspresi Kolagen tipe-1 (6th week) |
|---|---|---|---|
| 1. | P0 (NOV + OST + CMC Na) | $11,8 \pm 2,28^{a}$ | $16,0 \pm 2,0^{d}$ |
| 2. | P1 (OV + OST + CMC Na) | $4,8 \pm 2,49^{b}$ | $7,0 \pm 1,87^{bc}$ |
| 3. | P2 (OV + OST + RLX) | $9,0 \pm 1,41^{c}$ | $12,0 \pm 1,58^{ae}$ |
| 4. | P3 (OV + OST + CQ ) | $8,4 \pm 1,34^{c}$ | $14,4 \pm 2,61^{de}$ |

a, b, c, d, e: Different superscripts indicate significant differences ($p <0.05$).

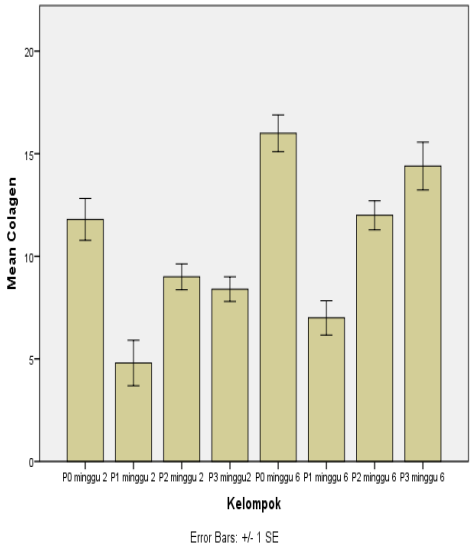


**Fig. 1:** Graphic of type I collagenexpression at the 2nd week and the 6th week at all groups of treatments.

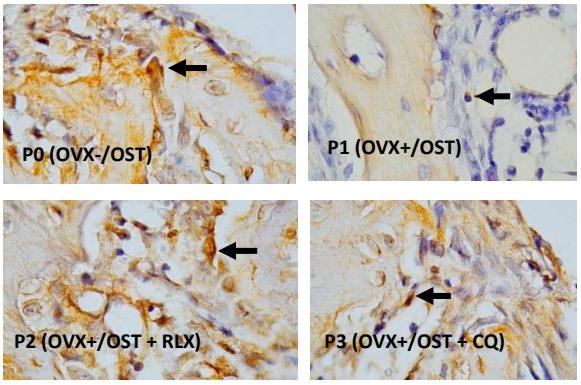


**Fig. 2:** The examination result of immunohistochemistry expression of type I collagen at the 2nd weeks at all group of treatments, the arrows indicate cells expressing type I collagen (brown).

This proves that estrogen deficiency that occurs in ovariectomized rats are capable of causing disturbances of fracture healing because estrogen plays an important role in the proliferation of osteoblasts that are responsible for the formation of collagen. The result of immunohistochemistry type I collagen could be seen in Figure2 and 3.

The results of this study also showed that the therapy *Cissus quadrangularis*(CQ) in rats ovariectomized, gives a good result in the increased expression of type I collagen, it can be seen that although the 2nd week in group P3 is not significantly different from the P0, but on the observation of the week 6th demonstrate its significant difference is the result of the expression of type I collagen on P3 is almost equal to P0. *Cissus quadrangularis* (CQ) is a plant that has osteogenic potential that is capable of increasing the proliferation and differentiation of osteoblasts. It is hypothesized that the *CQ* extract can accelerate the enzyme secretion by the osteoblasts, which accelerates the process of mineralization either by increasing the local concentration of inorganic phosphate or activating the collagen fibers to induce deposition of calcium salts[11].

Phytoestrogen contains in the *CQ*are isoflavones, lignin, coumestan, triterpene, glycoside, and acyclic are able to bind to the beta receptor of estrogen in osteoblast and could stimulate the proliferation of osteoblast [12].The exact molecular mechanism involved in *CQ* promoted osteogenesis remains to be explored. However, some evidence suggests that Wnt signaling may be involved. This pathway has been shown to play a significant role in the control of osteoblastogenesis and bone formation[10].It has been established that this signaling pathway regulates many aspects of osteoblast physiology including commitment, differentiation, bone matrix formation/mineralization and apoptosis as well as its coupling with osteoclastogenesis and bone resorption. Therefore, it is reasonable to suggest that the active constituents of CQ may stimulate the proliferation and differentiation of MSCs and promote new bone formation through the Wnt–LRP5–ß-catenin signaling pathway for pre-osteoblast formation[13].

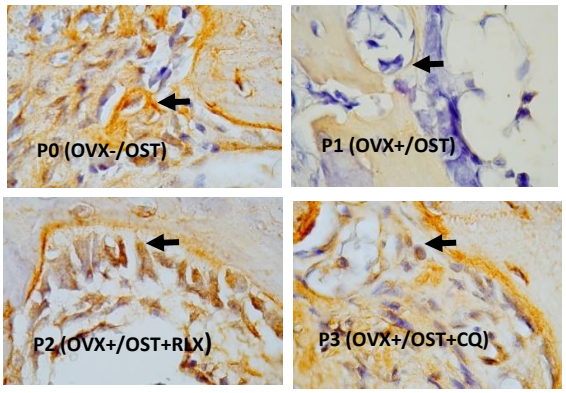


**Fig. 3:** The examination result of immunohistochemistry expression of type I collagen at the 6thweeks at all group of treatments,the arrows indicate cells expressing type I collagen (brown)

Collagen is an organic matrix produced by osteoblasts, so that if the proliferation and differentiation of osteoblasts good, then it will also be good collagen production. Collagen is a protein important for bone, which makes up almost 95% of the organic component of bone and is responsible for the flexibility of bone.

Several studies demonstrate that bone strength ismainly determined by tissue mass and stiffness, which is determined by the mineral phase, whereas thecollagen matrix contributes mainly to bone toughness [14]. Another in vitro study demonstrated the important role of the organic matrix for the mechanical properties of bone. Fantner et al. [15] reported that heat-degradedthe organic matrix and, consequently, changed the microfracture appearance and behavior of trabecular bone, resulting in decreased elasticity and in toughness.

The poorer mechanical properties found in the osteoporotic healing callus may bewell explained by both BMD data and histological findings, i.e., theinferior composition in anorganic matrix, especially collagens in callus. In fibrous callus, the early stage offracture healing, the collagen in the callus is predominantly typed III [16-18]. In the cartilaginous callus stage following the fibrous callus formation, however, the collagen was reported predominantly type II [16-17], secreted by chondrocytes, which laterbecame hypertrophic and secreted type-X collagen that was closely related to the mineralization of cartilage.With the progress of endochondroossification, type-II collagen decreased and type-I collagen secreted by osteoblasts became the predominant structural protein and about 90%of overall organic components in the new bone, which demonstrated stronger resistance to mechanical loading with healing over time[16-17]. This also suggested that the maturity of the callus could be evaluated by determining the ratio of the different types of collagens. In addition, the orientation of collagen fiber arrangement is also important, which defines a position for hydroxyapatite to deposit. The ideal mechanical

properties of bone depend on the highly organic combination of the types of collagens and the orientation of collagen fiber arrangement [16].

The concluded that *Cissus quadrangularis* extract can increase the expression type I collagen on femoral fractures in ovariectomized rats so that they were has a large impact on the mechanical properties of bone and stronger resistance to mechanical loading with healing over time

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## References

[1] Giannoudis P., Tzioupis C., Almalki T., Buckley R. Fracture healing in osteoporotic fractures: Is it really different? A basic science perspective. *Injury, Int. J. Care Injured*; (2007). 38S1, S90—S99

[2] Meyer RA, Tsahakis PJ, Martin DF, Banks DM, Harrow ME, Kiebzak GM. Age and ovariectomy impair both the normalization of mechanical

properties and the accretion of mineral by the fracture callus in rats. *J Orthop Res* (2001);**19**:428–35.

[3] Harada S, Rodan GA. Control of osteoblast function and regulation of bone mass. *Nat.*, (2003).423: 349-355

[4] Lukiswanto BS, Arifin MZ, Yudaniayanti IS. Expression of TGF-β1 During Fracture Repair in Ovariectomized Rats with Therapy of *Cissus Quadrangularis* Extract. *J Phys Pharm Adv*, (2015). 5(11): 779-786

[5] Mehta M, Kaur N, Bhutani KK. Determination of marker constituents from *Cissus quadrangularis*Linn and their quantitation by HPTLC and HPLC. *Phytochem Anal* (2001);12:91‑5.

[6] Jakikasem S, Limsiriwong P, Kajsongkarm T, Sontorntanasart T. Phytochemical study of *Cissus quadrangularis*. *Thai J Pharm Sci*. (2000);24:5

[7] Carrin SV, Garnero P, Delmas PD. The role of collagen in bone strength. *Osteoporos Int* (2006) 17: 319–336

[8] Adam SK, Das S, Othman F, Jaarin K. Fresh soy oil protects against vascular changes in an estrogen-deficient rat model: an electron microscopy study. *Clin.*, 64 (2009): 1113-9. DOI: 10.1590/S1807-5932200 9001100012.

[9] Flecknell P. *Laboratory Animal Anaesthesia*. 3rd Ed (2009). Elsevier Inc., London. 183-186

[10] Potu BK, Bhat KMR, Rao MS, Nampurath GK, Chamallamudi MR, Nayak SR. Petroleum ether extract of Cissus quadrangularis (Linn) enhances bone marrow mesenchymal stem cell proliferation and facilitates osteoblastogenesis. Clin., 64(2009): 993-998.

[11] Soumya S,.Sajesh KM, Jayakumar R, Nair SV, Chennazhi KP. Development of a phytochemical scaffold for bone tissue engineering using Cissus quadrangularis extract. *J. Carbohydrate Polymers* 87. (2012);1787– 1795.

[12] Yamaguchi M. Isoflavone and bone metabolism: its cellular mechanism prevention role in bone loss. *J. Health Sci.* (2002), 48(3): 209-220.

[13] Yavropoulou MP, Yovos JG. The role of the Wnt signaling pathway in osteoblast commitment and differentiation. Hormones. (2007);6:279-94.

[14] Currey JD, Brear K, Zioupos P . The effects of aging and changes in mineral content in degrading the toughness of human femora. *J Biomech* (1996) 29:257–260

[15] Zioupos P.Human bone: factors affecting its biomechanical properties and the role of collagen. *J Biomater Appl* (2001) 15:187–229

[16] Ashhurst DE. Collagens synthesized by healing fractures. *Clin Orthop Relat Res* (1990).225: 273-283

[17] Stephen HL, Yang RS, AL-Shaikh R . Collagen in tendon, ligament, and bone healing.*Clin Orthop Relat Res* (1995).318:265-278

[18] Sandberg M, Hannu TA, Eero IV. Gene expression during bone repair. Clin Orthop Relat Res (1993).289:292-312

# Parameters Genetic of Fruit Component Characters on Snake Fruit (*Salacca* sp.)

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## Abstract

The objectives of this research were to estimate the genotypic and phenotypic variabilities, heritability, genetics advance, and genotypic and phenotypic correlation of fruit component characters on some snake fruit accessions. This research was conducted at Bintan regency, from 2008 to 2009 with a Completely Randomized Block Design, 31 treatments (accessions), and two replications. Snake fruit accessions used were the hybrids came from crossing between Pondoh, Gula Pasir, Gondok, Mawar and Kersikan parents and Gula Pasir, Gondok, Mawar as local varieties. The results showed that all of characters had broad phenotypic variability. The characters of fruit weight, fruit length, fruit diameter had broad genotypic variability and high heritability. Fruit weight, fruit flesh thickness, and fruit diameter characters had high percentage of genetics advance.

Keywords: *Salacca* sp., variability; heritability, genetic advance, correlation

## 1. Introduction

Snake fruit (*Salacca zalacca* (Gaertner) Voss) is one of the tropical fruit crops native to Indonesian, hence, it is not surprising that this plant has high diversity in genetic resource spreading in most provinces of Indonesia. There are 20 species of Salacca genus found throughout the word and 13 species of them have spread around South East Asia particularly Indonesia (Mogea, 1990).

In general, consumers prefer snake fruit possessing thick flesh, sweet, slightly or not astringent, and long shelf life (Sunaryono, 1988). Varieties having such fully characters are hardly ever found in nature. For examples, Pondoh variety has superiority in sweet and non-astringent flavor, but its flesh is thin. Sidempuan variety produces big fruits, thick flesh, and 3-5 fruits per bunch, however its flesh is somewhat astringent, especially when unripe. Similarly, Bali variety has superiority in thick flesh, but slightly astringent. To meet demanded characters above, generating new varieties through assembling all characters should be attempted.

Indonesian Tropical Fruit Research Institute (ITFRI) has some hybrids and local accessions of snake fruit. These accessions had been evaluated on their growth both at the seedling stage and vegetatively stage (Hadiati et al., 2008; and Susiloadi et al., 2008). Their evaluation must be continued to generative stage in order to be selected their fruit qualities and quantities.

To selected their quality and quantities, are needed the information about inherity of some agronomies characters. This informations are needed to determine whether the observed characters can be used for the selection criteria to select the desired new genotype. Some genetic parameters that can be used as a justification for the selection can be effectively and efficienly are the genotypic variability, heritability, and genetic advance (Borojevic, 1990; Tampake and Luntungan, 2002). A character with broad genotypic variability will provide a high probability in

the selection of the best character.

The heritability of character has a major impact on the methods chosen for population improvement, inbreeding, and other aspects of selection. Single-plant selection may be effective for a character with high heritability (Fehr, 1987). Characters with high heritability will be inherited easily and the selection can be carried out on early generation (Hadiati et al., 2003 and 2009). Conversely, if the heritability is low, the selection of the next generation will be successful because of chences increase genetic diversity within populations (Falconer, 1970). Suprapto et al. (2007) stated that heritability determine the progress of selection. The greater a value of heritability, so the greater of genetic advance.

To date, how much genotypic and phenotypic variability, heritability, and genetic advance of snake fruit accession which be evaluated have not been revealed. The objectives of this research are to estimate genotypic and phenotypic variabilities, heritability and genetics advance of fruit component characters on some snake fruit accessions**.**

## 2. Materials and Methods

The research was carried out from 2008 to 2009 at Bintan regency with 350 m above sea level , the soil texture was sandy clay, and the rain fall was 2500 – 3500 mm/year. The research used Completely Randomized Block Design with 31 treatments (accessions), and two replications (years). The snake fruit accessions were hybrids and local varieties. The hybrids came from crossing between parents of Pondoh, Gula Pasir, Gondok, Mawar, Kersikan and the local varieties were Gula Pasir, Gondok, and Mawar varieties. All the plants were grown from seed, and six years old.

Characterization was performed on fruits of each plant, covering the weight of fruit (g), fruit length (cm), fruit diameter (cm), the thickness of flesh (cm), the edible portion of fruit (%), and Total Soluble Solid (TSS) (◦Brix). The thickness of flesh was the average of four sides of each fruit.

Genotypic and phenotypic variabilities were estimated by variance components analysis (Steel and Torrie, 1989). Genotypic and phenotypic variances were computed by Singh and Chaudhary (1979). To assess the genotypic and phenotypic coefficient of variations of a character were determined by Anderson and Bancroft (1952), *cit* Wahdah et al., 1996. A character had a broad variability if $\sigma_g^2 > 2\sigma_{\sigma_g^2}$ and $\sigma_f^2 > 2\sigma_{\sigma_f^2}$ and conversely.

Heritability (H) was estimated by using analysis of variance components and calculated based on the method of "broad sense" according to Allard (1960) and the criteria of heritability value according to Mc Whirter (1979):

$H = \frac{\sigma_g^2}{\sigma_f^2}$, where : H(%) : 0 – 20(low), 20 < H ≤ 50 (moderate), H > 50 (high).

Genetic advances (GA) is calculated according to Falconer (1989) and Natera *et al*. (2012). The percentage of genetic advance (GA %):

$GA(\%) = \frac{GA}{\bar{x}}$ x 100%, where GA = i H $\sigma_p$

Remarks:
GA = genetic advance
$\bar{x}$ = general mean
i = selection intensity
(estimate 5%: 2.06)
$\sigma_p$ = phenotypic standard deviations

Criteria of percentage of genetic advance according to Begum and Sobhan (1991) *cit* Bambang et al., (1998) : GA(%) : 0 - 7 % (low); GA(%) : 7.1 - 14 % (moderate) and GA(%) : > 14.1 % (high).

Coefficients of genotypic and phenotypic correlations of fruit weight with other component characters were calculated by following Singh and Chaudhary (1977).

Table 1. Means value, genotypic and phenotypic varians on some fruit component characters of snake fruit

| Characters | Means | $\sigma_g^2$ | $2\sigma_{\sigma_g^2}$ | Criteria | $\sigma_f^2$ | $2\,\sigma_{\sigma_f^2}$ | Criteria |
|---|---|---|---|---|---|---|---|
| Fruit weight (g) | 40.30 ± 9.89 | 91.1726 | 47.9107 | broad | 98.4827 | 47.771 | broad |
| Flesh thickness cm) | 0.72 ± 0.15 | 0.0101 | 0.0083 | narrow | 0.0160 | 0.0078 | broad |
| Fruit diameter (cm) | 4.18 ± 0.53 | 0.2079 | 0.1161 | broad | 0.2237 | 0.1153 | broad |
| Fruit length (cm) | 5.06 ± 0.71 | 0.1972 | 0.1885 | broad | 0.3536 | 0.1715 | broad |
| Total Soluble Solid (°Brix) | 19.13 ±1.27 | 0.1163 | 0.5564 | narrow | 0.8566 | 0.4154 | broad |
| Seed weight(g) | 3.56± 0.95 | 0.3351 | 0.3221 | narrow | 0.6036 | 0.2928 | broad |
| Edible portion (%) | 62.59 ± 10.02 | 16.7571 | 18.0726 | narrow | 33.1864 | 16.098 | broad |

Table 2. Heritability (H) and percentage of genetic advance (GA%) on some fruit component characters of snake fruit

| Characters | H (%) | Criteria | GA(%) | Criteria |
|---|---|---|---|---|
| Fruit weight | 92.58 | high | 45.26 | high |
| Flesh thickness | 63.27 | high | 22.18 | high |
| Fruit diameter | 87.52 | high | 21.48 | high |
| Fruit length | 55.76 | high | 13.32 | moderate |
| Total Soluble Solid | 13.58 | low | 1.36 | low |
| Seed weight | 55.51 | high | 23.98 | high |
| Edible portion | 50.49 | high | 9.65 | moderate |

*Remarks : H (%): 0 – 20 (low), 20 < H ≤ 50 (moderate) , H > 50 (high);*

*GA(%) : 0 - 7 (low), 7.1 - 14 (moderate ), and ≥ 14.1 (high)*

Table 3. Coefficient of genotypic correlation of fruit weight with other characters on snake fruit

| | Flesh thickness | Fruit diameter | Fruit length | Total Soluble Solid | Seed weight | Edible portion |
|---|---|---|---|---|---|---|
| Fruit weight | 0.89* | 0.96* | 0.89* | 0.81* | 0.13 | 0.46* |
| Flesh thickness | | 0.80* | 0.83* | 0.82* | -0.12 | 0.67* |
| Fruit diameter | | | 0.93* | 1.08 | 0.09 | 0.36 |
| Fruit length | | | | 0.54* | 0.13 | 0.98* |
| Total Soluble Solid | | | | | -0.31 | 0.87* |
| Seed weight | | | | | | -0.84* |

**Significant at P ≤ 0.05*

Table 4. Coefficient of phenotypic correlation of fruit weight with other characters on snake fruit

| | Flesh thickness | Fruit diameter | Fruit length | Total Soluble Solid | Seed weight | Edible portion |
|---|---|---|---|---|---|---|
| Fruit weight | 0.72* | 0.92* | 0.75* | 0.25* | 0.14 | 0.31* |
| Flesh thickness | | 0.83* | 0.51* | -0.07 | 0.09 | 0.57* |
| Fruit diameter | | | 0.62* | 0.36* | 0.17 | 0.44* |
| Fruit length | | | | 0.23 | 0.05 | 0.34 |
| Total Soluble Solid | | | | | -0.41* | 0.33 |
| Seed weight | | | | | | -0.58* |

**Significant at P ≤ 0.05*

## 3. Results

In Table 1 showed that there were the variations among fruit component characters. The largest variations were found in the character of the fruit weight and edible portion The accessions which be observed had the fruit weight on range of (40.30± 9.89g), and the edible portion of (62.59±10.02 %). The large variations in both characters would provide an opportunity to obtain a large fruit with a high edible portion. Snake fruit accessions were observed to have a high TSS average, *i.e* 19.13 ± 1.27 (°brix).

For all characters, the genotypic varians were higher in magnitude than phenotypic varians. The characters of fruit weight, fruit diameter and fruit length had the broad genotypic variability, while the others characters had the narrow genotypic variability. All characters had broad phenotypic variability. The characters that had broad the genotypic and phenotypic variability were fruit weight, fruit length and fruit diameter (Table 1). Heritability (broad sense) varied from 13.58% (total soluble solid) to 92.58% (fruit weight). Except for total soluble solid character, all other characters gave high heritability estimates (Table 2).

The percentage of genetic advance with 5% of selection intensity ranged from 1.36 (total soluble solid) to 45.26% (fruit weight) (Table 2). The characters that had a high percentage of genetic advanced were fruit weight, flesh thickness fruit diameter and seed weigh. Characters that had a moderate percentage of genetic advanced were fruit length, and edible portion. Overall, coefficients of genotypic correlations were higher than phenotypic correlations.

## Conclusion

Selection of the character fruit weight and fruit diameter could be done effectively and efficiently at early generation, because they had a broad genotypic and phenotypic variabilities, high heritability and high percentage of genetic advance.

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## References

Allard RW (1960). Principles of Plant Breeding. John Wiley and Sons, Inc. New York. Chichestor-Brisbane-Toronto- Singapore.

Anbanandan V, Saravanan K, and Sabesan T (2009). Variability, heritability, and genetic advance in rice (*Oryza sativa* L.). *Intl. J. Plant Sci.* 3(2) : 61 – 63.

Bambang H, Purwati RD, Marjani, dan Budi US ( 1998). Parameter genetik komponen hasil dan hasil serat pada aksesi kenaf potensial. *Zuriat.* 9(1) : 6 – 12.

Bhadru D, Rao VT, Mohan YC, and Bharathi D (2012). Genetic variability and diversity studies in yield and its component traits in rice (*Oryza sativa* L.) . *Sabrao Journal of Breeding and Genetics.* 44(1) : 129 – 137.

Falconer, DS (1989). Introduction to Quantitative Genetics. ELBS. Longam. London. 468p.

Fehr (1987). Principles of Cultivar Development.Theory and Technique. Volume I. Iowa State University. London. 536p.

Hadiati S, Murdaningsih HK, Baihaki A, dan Rostini N (2003). Parameter genetik karakter komponen buah pada beberapa aksesi nenas. *ZURIAT.* 14(2): 53 – 58**.**

Hadiati S., A. Susiloadi, dan T. Budiyanti. 2008. Hasil persilangan dan pertumbuhan beberapa genotipa salak. *Buletin Plasma Nutfah*. 14(1): 26-32.

Hadiati S., A. Susiloadi, dan T. Budiyanti. 2009. Seleksi genotipa salak manis berdasarkan aktivitas tanin pada fase bibit. *Agrivita*. 31(1): 19 – 24.

Jaiswal HK, Srivastava AK, and Dey A (2007). Variability and association studies in indigenous aromatic rice (*Oryza sativa* L.). *Oryza*. 44(4) : 351 – 353.

Mc. Whirter KS (1979). Breding of Cross Pollinated Crops. P. 79-110. *In.* R. Knight (eds). Plant Breeding. AA VCS, Brisbane.

Mogea J (1990). The Salak Palm Species in Indonesia. *Voice of Nature*. 85: 42&62.

Natera JRM, Rondon A, Hernandez J, and Merazo JF ( 2012). Genetic studies in upland cotton. III.

Genetic parameters, correlation and path analysis. *Sabrao Journal of Breeding and Genetics.* 44(1) : 112 – 128.

Nandariyah, Purwanto E, Sukaya, dan Kurniadi S ( 2000). Pengaruh tetua jantan dalam persilangan terhadap produksi dan kandungan kimiawi buah salak Pondoh Super. *Zuriat.* 11(1) : 33 – 38.

Prasad B, Parway AK, Biswas PS (2001). Genetic variability and selection criteria in fine rice (*Oryza sativa* L.) *Pak.J.Biol.Sci*. 4: 1188 – 1190.

Sarkar KK, Bhutia KS, Senapathi BK, and Roy SK (2007). Genetic variability and characters association of quality traits in rice (*Oryza sativa* L.). *Oryza*. 44(1) : 64 -67

Singh RK, and Chaudhary BD (1979). Biometrical Methods in Quantitative Genetic Analysis. Kalyani Publisher, New Delhi. p : 52 - 53.

Singh SK, Singh CM, and Gil GM (2011). Assessment of genetic variability for yield and its component character in rice. *Research in Plant Biol.* 1(4) : 73-76.

Steel RGD, dan Torrie JH (1989). Prinsip dan Prosedur Statistik (terjemahan oleh Bambang Sumantri ). P.T. Gramedia. Jakarta.

Sunaryono H (1988). Perkembangan Salak. Ilmu Produksi Tanaman Buah-buahan. Sinar Baru, Bandung.

Susiloadi A, Hadiati S, dan Budiyanti T (2008). Keragaan pertumbuhan beberapa aksesi salak di Kab. Kampar. *J. Agritek*. 16 (5) : 943-948.

Suprapto, Narimah, dan Kairudin MD (2007). Variasi genetik, heritabilitas, tindak gen, dan kemajuan genetik kedelai (*Glycine max* Merr. ) pada ultisol. *Jurnal Ilmu-ilmu Pertanian Indonesia.* 9(2) : 183 – 190.

Tampake H, dan Luntungan HT ( 2002). Pendugaan parameter genetik dan korelasi antar sifat-sifat morfologi kelapa (*Cocos nucifera*, Linn). *Jurnal Littri* . 8(3):97 – 102.

Wahdah R, Baihaki A, Setiamihardja R, dan Suryatmana G (1996). Variabilitas dan heritabilitas laju akumulasi bahan kering pada tanaman kedelai. *Zuriat*. 7(2) : 92 – 98.

# Genetic Variability of Indonesian Papaya (*carica* spp.) as Revealed by RAPD (Rapid Amplified Polymorphic DNA)

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## Abstract

Indonesia was not the origin of papaya papaya (*Carica* spp.), however due the open pollination, it had many papaya accessions. RAPD (*Rapid Amplified Polymorphic DNA*) is one of the methods which can be used to examine the genetic variability of Indonesian papaya. The 14 RAPD markers were used to revealed the genomic profilling of the 23 papaya accession from the collection of Indonesian Tropical Fruit Research Institute. The gel image suggested 90 out of 119 polymorphic bands with size 200-2200 kb. The bands were scored both with BioDoc software and manually to produce binary file and later analyzed with NTSys Pc 2.10x to revealed the dendogram, whereas the Winboot software was used to determine the bootstrap values of the dendogram's branches. The analysis demonstrated that the Dice-Sorensen coefficient similarity of the studied papayas was ranged from 0.71 – 0.98. The UPGMA and PCA (Principal Component Analysis) analyses revealed that the papaya germplams in this study were clustered on six distinct groups, four of them had a single member namely Sicincin Panjang, Lokal Sumani, Cariso, and Carica. Eventhough the clustering on this study did not represent the morphology differences, these results can be used as a reference on papaya crossbreeding study to obtain consumers preferred variety.

Keywords: Carica spp., genetic variability, PCA, RAPD, UPGMA

## 1. Introduction

Although, Indonesia is not the said origin of papaya, due to the nature, papaya in Indonesia had been diversed. The genetic polymorphism of papaya is the most important basis for the variation of plants phenotypes [1]. Information of the diversity within a particular crop species is required to comprehend evolutionary relationships, utilize effective approach for preservation of genetic resources, and effectively directing the breeding and selection for crop improvement [2].

The Indonesian Tropical Fruit Research Institute (ITFRI) collection of papaya germplasms was developed from exploration, hybridization, and inbred line activities. The ITFRI has launched a number of commercial papaya varieties, including Merah Delima, Agri Solinda, Carvita Agrihorti, and several new variety candidates. The assembly of papaya new variety was developed based on consumers prefferences, namely sweet taste, thick and orange fruit flesh, high productivity, and resistant to environment stress.

The utilization of germplasms to improve agronomy traits of the chosen accessions is conducted based on accurate genetic information, thus the precise individual plant as the breeding materials can be determined. The commonly method to access plant genetic data is RAPD (Random Amplified Polymerase DNA), which is adventages in rapid, relatively easy and simple technique, thus can be operated in a undeveloped laboratory with minimum

equipments. The result of RAPD analysis applicale in confirmation of genetic linkage [2] and diversity [4], tolerant and quatitatives traits [5], somaclonal variation [6,7], genotoxic effect [8], also sex determination in seedling [9].

Papaya is one of the popular fruits in the world, thus studies related to thid tropical fruit had been conducted rapidly, including its genetic assesment which is accomplish the whole genome sequensing [10]. The genetics research of papaya were also related to sex determination [11,12], gene characterization [13], and genome wide analysis [14,15]. In addition, the papaya genetic diversity had also been widely studied, which were included the use of microsatellite markers to asses polymorphism [16,17,18], the realtionship among papaya and its relatives [19], and among the papaya local germplasms [20,21,22,23,24]. However, in our concern only few of the genetic papaya research which were involved Indonesian papaya germplasm, thus this study was conducted to assess the genetic diversity of said fruit.

## 2. Material and Methods

*2.1. DNA extraction*

DNA extracted from papaya leaves using the modified CTAB method [25]. A total of 100 mg the papaya leaves were ground with 1.5 ml of extraction buffer, 1% β-mercaptoethanol, and 10 mg of PVP-10 to form a paste. Papaya leaves paste durian samples were then transferred into a 2 ml centrifuge tubes and incubated at 65$^{o}$C for 60 minutes. Protein were degraded three times with 500 ml of chloroform: Isoamyl-alcohol (24: 1), and then centrifuged at 12,000 rpm for 10 minutes. The DNA was precipated with the addition of 500 ml of cold iso-propanol, whereas the RNA was degraded with 2 mg/ml RNAse, then centrifuged at 12,000 rpm for 10 minutes. The formed DNA pellets were air dried, rinsed with 70% ethanol, and dissolved in 50 ml of TE buffer. DNA was quantified using nano-drop equipment.

*2.2. RAPD amplification, electrophoresis, and data analysis*

The total of 14 primers used in this study. RAPD PCR reaction was performed with Taq PCR reaction mixture 4.25 µl (KAPPA) with 1 µl of RAPD, 1 µl (10 ng) of sample DNA, and dd$H_2O$ to a final volume of 12.5 µl reaction. PCR was performed by 45 cycles with the following programs: pre denaturation 95$^{o}$C for 3 minutes, denaturation 95$^{o}$C for 15 seconds, annealing 36$^{o}$C for 15 seconds, extension 72$^{o}$C for 5 seconds, and final extension: 72$^{o}$C for 10 minutes. The PCR formula and programs were optimized prior the real RAPD test to ensure the reproducibility of the test as suggested by Ghazi et. al. [26] (2013). The whole experiments were run in triplicate to check for the reproducibility.

The primers selection of originally 30 primers were conducted in order to find the suitable primers which were able to showed the molecular diversity on individual level. The primers selection was conducted by using 5 samples. As much as 14 primers which demonstrated polymorphic bands were chosen for further investigation, which were RAPD1(GCGGGTGGAA),RAPD2(GTTTCGCTCC), RAPD3 (GTAGACCCT), RAPD4 (AAGAGCCCGT),RAPD5(AACGCGCAAC),RAPD6(CCCGTCAGCA),OPA01(CAGGCCCTTC),OPA02(TGCCGAGCTG),OPA11(CAATCGCCGT),OPA14(TCTGTGCTGG),OPC01(TTCGAGCCAG),OPC04(CCGCATCTAC), OPN09 (TGCCGGCTTG), and OPY15 (AGTCGCCCTT).

DNA amplification product was separated by electrophoresis at 50 V for 20 minutes. The band pat terns were scored manually and automated using software BioDoc Analyzer. The Dice-Sorensen coefficient of similarity and the UPGMA method were used to construct the dendogram. Relationship among the hybrids and parent were investigated using Principal Co-ordinates Analysis based on Eigen value method. All of the calculation was conducted

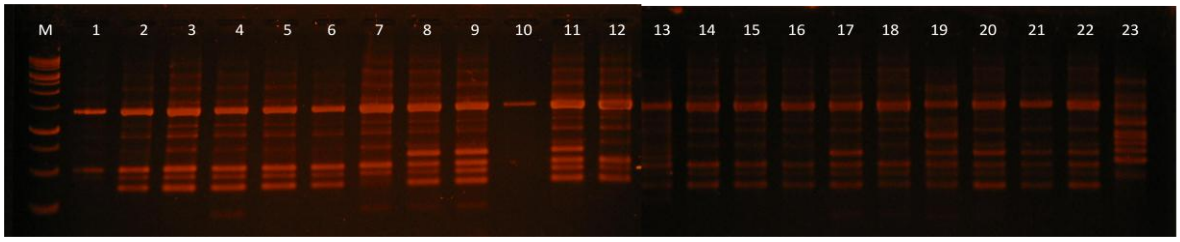
using the NTSYSpc 2.10x software [27], whereas the bootstrap analysis was conducted using Winboot software [28].

## 3. Result and Discussion

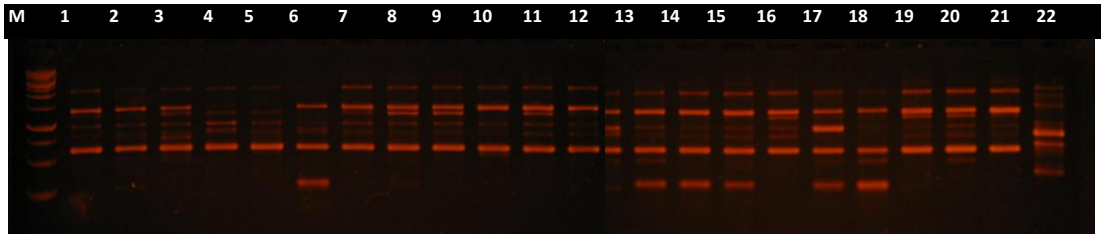
To our concern, this paper was one of the few publish study of the genetic variability of Indonesian papaya. The 23 papaya accessions on this study were the collection of Indonesian Tropical Fruit Research Institute which conduct papaya breeding program to develop new variety. Thus, the result of this study will be used as refrence to select the appropriate parent for the future crossbreeding as well as to evaluate the hybrid from the activity.

The RAPD was chosen to asses the papaya genetic variability due to its simplicity, yet produce accurate result. Among the 30 RAPD primers tested, 14 primers result 119 bands, included 90 polymorphic bands (75.6%). Each primer amplified 4-9 band with an average 8.5 band per primer and size of 2000-2200 kb size. Some representatives gel electrophoresis of papaya samples with some RAPD primers were performed in Figure 1.

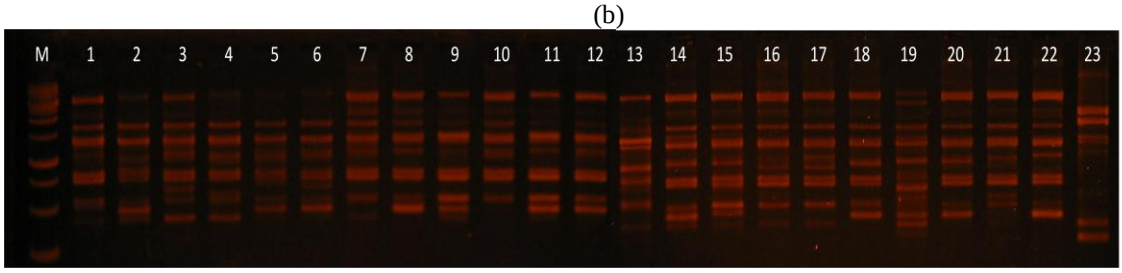
The gel image in Figure 1(a) showed that a 1500 bp monomorphic band was appeared, whereas the rest of the bands ware polymorhic. The similar phenomenomes also occured in the rest of the gel.




(a)




(b)




(c)

Fig 1. RAPD-PCR Gel electrophoresis of 23 papaya accession using primers; (a) OPA02, (b) RAPD3, and (c) OPY15 (M = 1 kb DNA marker; 1= Solinda, 2= Merah Delima, 3= Daun Jarak, 4= Carisya, 5= Dampit, 6= Tangkai Ungu, 7= BTK3, 8= BT2, 9= BT3, 10= Sicincin Panjang, 11= 2X1, 12= 1XD, 13= Lokal Sumani, 14= G18, 15= G30, 16= G28, 17= Red Lady, 18= Sunrise, 19= Cariso, 20= BT4, 21= G10, 22= G15, 23= Carica).

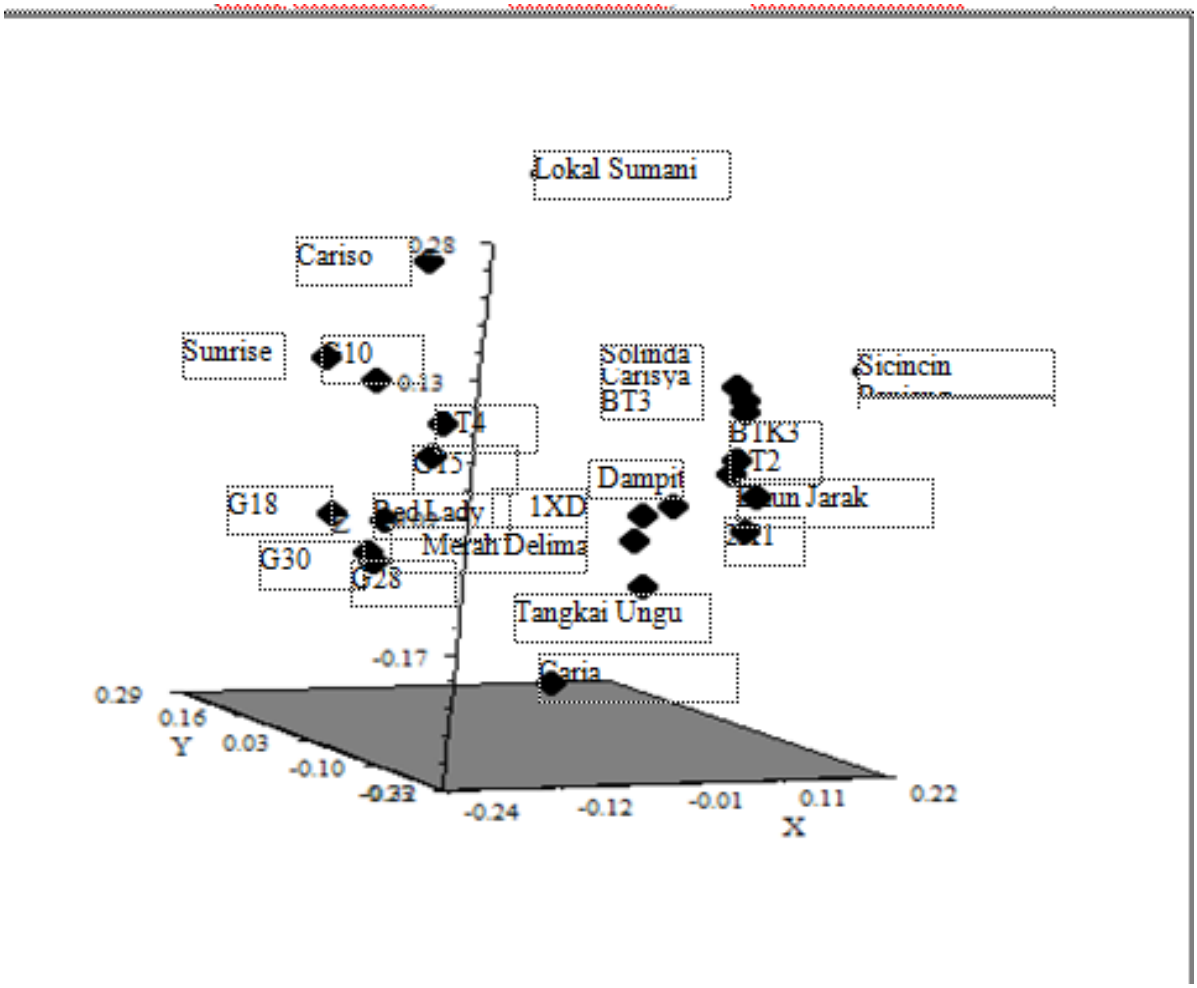



Figure 2. 3-D plot of 23 papaya accessions based on Eigeunvector analysis.

The Dice-Sorensen similarity test revealed that the further related samples (0.71) were Sicincin Panjang and Carica, whereas the closest related samples were Solinda dan BTK3, G28, and G30 (0.98) or showed moderate level of genetic diversity, which was also occured on Indian papaya genotypes (Saran et al. 2015).

However, other repot on Indonesian papaya genetic diversity was conducted on 20 accession from the collection of Pusat Kajian Hortikultura Tropika (Bogor Agriculture Institute) using 6 RAPD primers. The previous RAPD analysis showed that the similarity coefficient among the genotype was range from 0.00 to 1.00 which was performed low genetic diversity [29].

The dendogram construct following the UPGMA analysis was represented in Figure 2. The Pricipal Component Analysis (PCA) comfirmed that the clustering of the samples in the dendogram. The three-D plot of the PCA based on the Eigeun vector was shown in Figure 3.

Based on 0.87 coefficient similarity, the accessions can be clustered in six different group, which was included four groups with single member. The Solinda dan BTK3, both have yellow flesh color were grouped in the same cluster indicated that the flesh color was morhological marker in papaya characterization study.

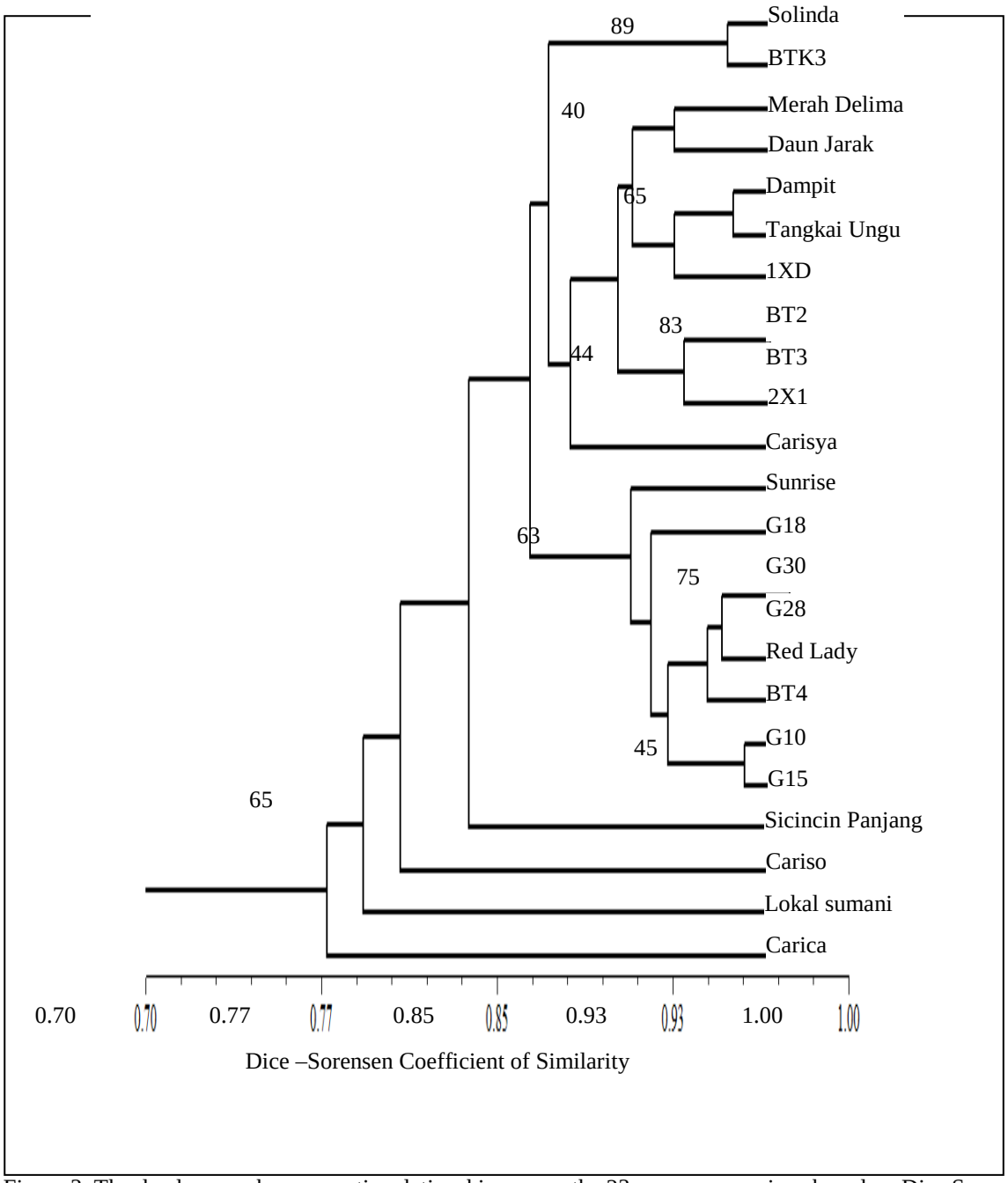



Figure 3. The dendogram shows genetic relationship among the 23 papaya accessions based on Dice Sorensen coefficient of similarity and UPGMA analysis

Although previous study stated thet there was no significant correlation between morphology and ganatic characteristics of papaya [20,29], the flesh color on this study was appear to be one of the morphological color in papaya which was substantiated by the RAPD marker as well as reported by Saran et al. [24].

The clustering in Solinda and BT3 also implied that eventhough samples comes from different seeds, they showed similar genetic characteristics. This fact also occured in the case of BT2 and BT3, G28 and G30 which

implied that the papaya propagation using seeds produced similar genetic characteristics. It was also illustrated that small chance of cross breeding in the papaya propagation through seeds.

Other samples, which were Sicincin panjang, Lokal Sumani, Cariso, and Carica were group as a single member cluster which represnted their morphology which was different from the rest of samples. The Sicincin Panjang, is papaya local varieties from Padang Pariaman Regency which very big and long fruit, the Lokal sumani is local varieties from Solok Regency showed big fruit, Carica is local Variety of Dieng Mountain of Yogyakarta Regency. The carica (*Vasconcellea* spp.) is a agro-economic highland papaya which was despite the similar morphology, was not the closest relative of *Carica* [30]. The local varieties tend to self breeding, thus resulted offspring with more homozygos alleles and unique characteristics [31].

## Conclusion

Indonesian papaya genetic variability was considered as moderate. The Sicincin panjang, Lokal Sumani, and Cariso were important germplasms which might contribute as a source of desire characteristics in papaya breeding program.

## Acknowledgments

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## References

[1] P.H. Moore," Phenotypic and genetic diversity of papaya," In: Ming, R. and P.H. Moore (eds.) Genetics and genomics of papaya, Plant genetics and genomics: Crops and models 10, Springer Science+Business Media, New York pp: 35-45. 2014

[2] Y.K. Chan, "Breeding papaya (*Carica papaya* L.)," In: S.M. Jain, P.M. Priyadarshan (eds.), Breeding plantation tree crops: Tropical species, Springer Science+Business Media, LLC, New York, pp: 121-159, 2009.

[3] S.Venkat, P. Bommisetty, M.S. Patil, L. Reddy, and A.Chennareddy, " The genetic linkage maps of Anthurium species based on RAPD, ISSR and SRAP markers," Scientia Horticulturae 178: 132-137, 2014.

[4] F. Nejatzadeh-Barandozi, M.R. Naghavi, S.T. Enferadi, A. Mousavi, Y. Mostofi, M.E. Hassani "Genetic diversity of accessions of Iranian *Aloe vera* based on horticultural traits and RAPD markers," Industrial Crops and Products 37: 347-351, 2012.

[5] A.G.A Khaled, M.H. Motawea, and A.A. Said, "Identification of ISSR and RAPD markers linked to yield traits in bread wheat under normal and drought conditions," Journal of Genetic Engineering and Biotechnology 13: 243-252, 2013.

[6] T. Dey, S. Saha, and P.D. Ghosh, "Somaclonal variation among somatic embryo derived plants: Evaluation of agronomically important somaclones and detection of genetic changes by RAPD in *Cymbopogon winterianus*," South Afican Journal of Botany 96: 112-121, 2015.

[7] M. Stanisic, M. Raspor, S. Ninkovic, S. Milosevic, D. Calic, B. Bohanec, M. Trivfunovic, M. Petric, A. Subotic, and S. Jevrimovic, "Clonal fidelity of *Iris sibirica* plants regenerated by somatic embryogenesis and organogenesis in leaf-base culture — RAPD and flow cytometer analyses," South Afican Journal of Botany 96: 42-52, 2015.

[8] O. Aksakal, F.G. Ertuk, S. Sunar, S. Bozari, and G Agar, " Assessment of genotoxic effects of 2,4-dichlorophenoxyacetic acid on maize by using RAPD analysis," Industrial Crops and Products 42: 552-557, 2013.

[9] G. Vaidya and D.G.R. Naik, "Molecular identification of sex in *Simarouba glauca* by RAPD markers for crop improvement strategies," Biotechnology Reports 4: 56-59, 2014.

[10] R. Ming, Q. Yu, P.H. Moore, R.E. Paull, N.J. Chen, M. Wang, M.A. Schuler, J. Jiang, and A.H. Paterson, "Genome of papaya, a fast growing tropical fruit tree," Trees Genetics & Genomes 8: 445-462, 2012.

[11] R. Ming, Q. Yu, and P.H. Moore, "Sex determination in papaya," Seminars in Cell & Developmental Biology 18: 401–408, 2007.

[12] R. Aryal and R. Ming, "Sex determination in flowering plants: Papaya as a model system," Plant Science 217-218: 56-62, 2014.

[13] R.E. Paull, B. Irikura, P. Wu, H. Turano, N.J. Chen, a. Blas, J.K. Fellman, A.R. Gschwend, C.M. Wai, Q. Yu, G. Presting, M. Alam, and R.

Ming, "Fruit development, ripening and quality related genes in papaya genome," Tropical Plant Biology 1: 246-277, 2008.

[14] B.W. Porter, M. Paidi, R. Ming, M. Alam, W.T. Nishijima, and Y.J. Zhu, "Genome-wide analysis of *Carica papaya* reveals a small *NBS* resistance gene family," Molecular Genetics and Genomics 281: 609-629, 2009.

[15] K. Liu, C. Yuan, H. Li, Y. Yang, C. Shen, and X. Zheng, "Genome-wide identification and characterization of *auxin response factor* (*ARF*) family genes related to flower and fruit development in papaya (*Carica papaya* L.)," BMC Genomics 16: 901, 2015.

[16] E.J. De Oliviera, V.B.O. Amorim, E.L.S. Matos, J.L. Costa, M.D.S. Castellen, J.M. Padua, and J.L.L. Dantas, "Polymorphism of microsatellite markers in Papaya (*Carica papaya* L.)," Plant Molecular Biolology and Reproduction 28:519-530, 2010.

[17] S. Sengupta, B. Das, M. Prasad, P. Achary, and T.K. Ghose, "A comparative survey of genetic diversity among a set of Caricaceae accessions using microsatellite Markers," Springer Plus 2: 345, 2013.

[18] G.A.F. Oliviera, J.L.L. Dantas, and E.J. Oliviera, "Informativeness of minisatellite and microsatellite markers for genetic analysis in papaya," Genetica 143: 613-631, 2015.

[19] F.A. Carvalho and S.S. Renner, "A dated phylogeny of the papaya family (Caricaceae) reveals the crop's closest relatives and the family's biogeographic history," Molecular Phylogenetics and Evolution 65:46-53, 2012.

[20] J. Ocampo, J., G.C. d'Eckenbrugge, S.B. Ruyere, L.D.L. de Bellaire, and P.O. Llitrault, " Organization of morphological and genetic diversity of Caribbean and Venezuelan papaya germplasm," Fruits 61: 25-37, 2006.

[21] P.O. Aikpokpodion, "Assessment of genetic diversity in horticultural and morphological traits among papaya (Carica papaya) accessions in Nigeria," Fruits 67: 173-187, 2010.

[22] J.E. Brown, J.E., J.M. Bauman, J. F. Lawrie, and O.J. Rocha, "The Structure of morphological and genetic diversity in natural populations of *Carica papaya* (Caricaceae) in Costa Rica," Biotropica 44(2): 179-188, 2012.

[23] Madarbokus, S and V.M. Ranghoo-Sanmukhiya. 2012. Identification genetic diversity among papaya varieties in Mauritius using morphological and molecular markers. International Journal of Life Sciences Biotechnology and Pharma Research 1(4); 153-163.

[24] P.L. Saran, R. Chaudhary, I.S. Solanki, P. Patil, and S. Kumar, "Genetic variability and relationship studies in new Indian papaya (*Carica papaya* L.) germplasm using morphological and molecular markers," Turkish Journal of Agriculture and Forestry 39: 310-321, 2015.

[25] J.L. Doyle and Doyle J.L, "A rapid DNA isolation procedure for small quantities of fresh leaf tissue," Phytochemistry Bulletin 19: 11-15, 1987.

[26] F. Ghazi, Z. Benmechernene, M. Kihal, and G.C. Gurakan, "The reproducibility of random amplified polymorphic DNA (RAPD) profiles of *Streptococcus thermophilus* strains with XD9, M13, and MOD primers," African Journal of Biotechnology 12(44): 6245-6252, 2013.

[27] F.J. Rohlf, "NTSYSpc: Numerical taxonomy and multivariate analysis sistem, ver 2.10x," Exeter Publishing Ltd., Setauket, NY, 2004.

[28] I.V. Yap and R.J. Nelson, "Winboot: A program for performing bootstrap analysis of binary data to detremine the confidence limits of UPGMA-based dendogram," IRRI Discussion Paper Series No.14. IRRI, Manila: 32 pp, 1996.

[29] R.Y.B. Galingging, "Analisis keragaman 20 genotipe pepaya berdasarkan penanda morfologi dan RAPD (Random Amplified Polymorphic DNA). Master Thesis. Institute Pertanian Bogor, Bogor: xv + 65 pp, 2005.

[30] G.C. D'Eeckenbrugge, R. Drew, T. Kyndt, and X. Scheldeman, "*Vasconcellea* for papaya improvement," In : Ming, R. and P.H. Moore (eds.) Genetics and genomics of papaya, Plant genetics and genomics: Crops and models 10, Springer Science+Business Media, New York pp: 46-79, 2014.

[31] A.Rodriguea-Burruezo, J. Prohens, and F. Nuez, "Performance of hybrids between local varieties of eggplant (*Solanum melongena*) and its relation to the mean of parents and to morphological and genetic distances among parents," Europ.J.Hort.Sci., 73 (2): 76–83, 2008.

# MEDICINE

# The Effects of Oxidation And Thermolysis Reaction on a-Mangostin Content in the Ethyl Acetate Extract of Mangosteen Rind (*Garcinia mangostana* L.) by High Performance Liquid Chromatography

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## Abstract

Mangosteen (*Garcinia mangostana* L) rinds contain high concentration of xanthones such as α-, and γ-mangostin. The α-mangostin-rich extracts have been used widely in nutritional supplements, herbal cosmetics and pharmaceutical preparations. This research aimed to determine the effects of oxidation and thermolysis reaction on α-mangostin content in the ethyl acetate extract of the mangosteen rind (*Garcinia mangostana* L). High performance liquid chromatography method using a reverse phase column Waters® e 2695 µ boundapak (3.9 x 300 mm; 10 m), mobile phase of acetonitrile and 0.1% phosphoric acid (80: 20) at the flow rate of 0,8 mL min-1 with a UV detection at 243 nm using an auto sampler system, injection volume of 20 µL with retention time of α-mangostin was 6.3 min. The method was validated for linearity, precision, accuracy, limit of detection (LOD), and limit of quantitation (LOQ). The linearity of the proposed method was found in the range of 1.06–31.89 µg mL-1 with the correlation coefficient of 0.9999, sensitivity (LOD: 1.19 µg mL-1 and LOQ: 3.98 µg mL-1), intraday and interday precision RSD < 2%, accuracy (intraday between 99.93% - 101.05% and interday between 98.96% - 103.08%) and the recovery of 100.41% - 101.87%. The content of α-mangostin in ethyl acetate extract of mangosteen rind was 51.09 ± 0.22 % and α-mangostin contents in the ethyl acetate extract of mangosteen rind after oxidation and thermolysis were 48.06 ± 0.11 % and 44.04 ± 0.40 %, respectively. Thermolysis reaction produces a new compound with retention time (tr) of 4.74 min. The results of statistical analysis showed that there were significant differences between the contents of α-mangostin in ethyl acetate extract of mangosteen rind before and after an oxidation and thermolysis reaction ($p<0.05$).

Keywords : *Garcinia mangostana* L.; α-mangostin; HPLC; oxidation; thermolysis reaction

## 1. Introduction

Some research indicates that the mangosteen fruit rind is rich in antioxidants, especially anthocyanins, xanton, tannins and phenolic acids ([1], [2], [3]). Xanthones have a unique chemical structure composed of a tricyclic aromatic system (C6–C3–C6). Isoprene, methoxyl and hydroxyl groups located at various locations on the A and B rings, resulting in a diverse array of xanthone compounds. Xanthones are found in a select few higher plant families. At least 68 distinct xanthones have been identified in different parts of the *G. mangostana* plant with 50 being present in the fruit's pericarp at higher concentrations than in the aril or edible portion of the fruit [4]. The most abundant xanthones in the pericarp of mangosteen fruit are α- and γ-mangostin (Fig. 1) [5].

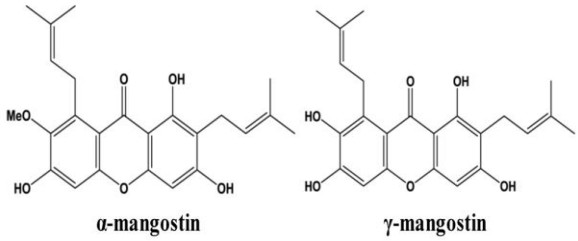



Fig.1. Chemical structure of α-mangostin and γ-mangostin

Alpha mangostin is a major compound contained in extract of mangosteen rind that serves as a marker compound from the extract of mangosteen rind. It also serves to determine the quality control of fruit mangosteen rind extract and preparations ([6],[7]). These compounds have various pharmacological activities such as antioxidants, anti-tumor, anti-inflammatory, anti-allergic, anti-bacterial and anti-fungal [8].

Alpha mangostin is derived polyphenolic compounds that have hydroxyl groups. This causes the hydroxyl groups in polyphenol compounds are very easily oxidized and very sensitive to light so that it will affect the stability of this compound [9]. Ethyl acetate is used as the solvent extraction because α-mangostin is a semipolar compound.

Quantitative analysis methods α-mangostin has been done by spectrophotometry UV and TLC densitometry ([10],[11]). Both methods are efficient in the solvents, sample number and time consuming, but lacking in accuracy and precision [12].

The proposed HPLC method promoted high precision, sensitivity and accuracy for quality control of extract of *G. mangostana* L. rind. This proposed method will be useful for quantitative analysis of α-mangostin in standardization and quality assessment of extract of *G.mangostana*L. rind for pharmaceutical uses [13]. Therefore, the aim of this study was to determine the effect of degradation reactions include oxidation and thermolysis reactions on contents of α-mangostin in the ethyl acetate extract of mangosteen rind by high performance liquid chromatography. The results of these studies are useful as a source of information for quality control of mangosteen rind extract preparations.

## 2. Material and Methods

### *2.1. Chemical and reagents*

Alpha-mangostin was purchased from Wuxi Gorunjie Natural-Pharma Co., Ltd (Jiangsu, China; purity of 90,3 %). The chemicals and solvents used in this experiment were acetonitrile (HPLC grade), ethyl acetate, methanol, and ortho phosphoric acid 85 %, hydrogen peroxide 3 % (analytical grade) were purchased from Merck. High purity aqua bidestilated water was obtained from Otsuka. Solvents used for the mobile phase were filtered through membrane filter (0.45-µm pore size) and degassed before use.

### *2.2. Instrumentation and chromatographic condition*

Spectrophotometer UV-Vis (Shimadzu® 1800 AV), HPLC (Waters e 2695®) with µ boundapak C-18 column (3.9 x 300 mm, i.d. 10 µm), autosampler(SIL-10ADvp), solvent degasser (DGU-14A), UV detector(SPD-10ADvp).The UV spectra were recorded in the 200–400 nmrange, with a PDA (Agilent 1100 HPLC system), and the quantification wavelength was set at 243 nm. The mobile phase was consisting of A (acetonitrile) and B ($0.1\%^{v}/_{v}$ H3PO4 in water), the elution program was isocratic at 80% (A) and 20% (B) for 12 min, the flow rate was maintained at 0.8 mL/min, and the injection volume was 20 µl.

### *2.3. Preparation of plant materials*

The mangosteen rinds were collected from Belimbing area, district of Kuranji, Padang, West Sumatra, Indonesia. The fruit rinds were separated from the edible part, chopped using an electric grinder, and dried at

60°C for 48 h,until its weight is constant. The dried sample was roughly ground and kept in a tight container protected fromlight.Dry powder finely macerated, with ethyl acetate. The ethyl acetate extract was filtered with Whatman filter paper no.1, evaporated with a rotary evaporator at a temperature <50 $^0$C to obtain a concentrated extract. The sample was prepared by accurately weighing 50mg of mangosteen extract into a 100-mL volumetric flask.Approximately 60 mL of methanol was added, and the solutionwas sonicated for 15 min. The solution was allowed to cool toroom temperature before being filled up to the final volume of100.0 mL. After centrifugation for ~ 10 min, 0.5 mL of the supernatantwas diluted to 10 mL, in a volumetric flask by acetonitrileand filtered through a 0.45-μm filter membrane beforeanalysis. Twenty microliters of the sample solution was directlyinjected into the HPLC column and separated under described chromatographic conditions

*2.4. Preparation of standards and calibration standard solution*

The standard stock solutions of α-mangostin were prepared bydissolving their accurately weighted compounds in methanol togive the solution a final concentration of 100 μg/mL, and storedat 4°C until use. These solutions were then serially diluted withmethanol to provide calibration standard solutions of 1.0, 2.0, 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0 μg/mL.

*2.5. Assay characteristics for method validation*[14].

*Specificity*

The specificity was determined by analysis of a solution containing10 μg/mL of α-mangostin both for standard and mangosteenpeel extract solution. Methanol was used as a control. Avolume of 20 μL was individually injected into the HPLC systempreviously described. The specificity was then performed by comparingthe retention times of α-mangostin in the chromatogramof the extract solution with those in the chromatogram of thestandard solution.

*Linearity and calibration curve*

Standard α-mangostin solutions in the 1.0, 2.0, 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0 μg/mL range were injected into the HPLC system. Six replicateanalyses were performed per day. The calibration curve wasanalyzed using the linear least-squares regression equation.Calibration curves were constructed by plotting peak areaagainst the concentration of standards. A correlation coefficientabove 0.99 was acceptable.

*Limits of detection and quantitation*

For the evaluation of the limits of detection (LOD) and quantitation(LOQ), a concentration sequence of the standards wasprepared by diluting standard solutions with methanol andwas then analyzed with the HPLC system. LOD and LOQ werebased on three times and ten times of signal-to-noise ratio,respectively.

*Accuracy and precision*

Intra- and inter-day precision and accuracy were evaluated atthree different levels of standard α-mangostin concentrations (5.0, 10.0, and 15.0 μg/mL). Intra- and inter-day assay precisionwere determined as relative standard deviation (RSD), and intraandinter-day assay accuracies were expressed as percentages oftheoretical concentration, as accuracy (%) = (found concentration/ theoretical concentration)×100. Intra-day assay involvedthree replicates per day and inter-day assay were performed onthree separate days.

*Recovery*

Three level differences of standard α-mangostin concentrationswere spiked in dried mangosteen peel extract sample withknown contents of α-mangostin, and the samples

were processed according to the "Preparation of plant materials" proceduremaking the final concentration of standards to be as 5.0,10.0, and 15.0 µg/mL. The three injections for each concentrationwere done per day over three different days. The recoveriesof α-mangostin were calculated as the following equation:

$$\text{Recover (\%)} = \frac{C_{obs} - C_{blk}}{C_{act}} \times 100$$

where: $C_{obs}$ is the observed concentration of α-mangostindetected in the sample solution (µg/mL). $C_{blk}$ is the concentrationof α-mangostin detected in mangosteen peel extract samplesolution without added standard α-mangostin solution (µg/mL).$C_{act}$ is the actual concentrations of standard α-mangostin solution(µg/mL).

*The oxidation and thermolysis reaction of α-mangostin in the ethyl acetate extract of mangosteen rind*

Forced degradation of α-mangostin was carried out under thermolytic, and oxidative stressconditions. Thermal (in a controlled-temperature oven at 80°C for 3 h) was preceded in the ethyl acetate extract. After degradation, stock solution was prepared by dissolving ethyl acetate extractin methanol to achieve a concentration of 500 µg/mL.From these solutions, aliquots were diluted with 50% methanolto achieve a concentration of 25 µg/mL.

For oxidative degradation, solution was preparedby dissolving α-mangostin in extract in a small volume ofmethanol, and later dropped with 3% hydrogen peroxide (3% $H_2O_2$) solution and heated at 80°C for 3 h.After degradation, the stock solution was prepared by dissolvingin methanol to achieve concentration of 500 µg/mL. From these solution, aliquot was diluted with 50% methanol to achieve a concentration of 25 µg/mL. The sample solution for oxidative stress was kept in a dark to prevent the effect of light.

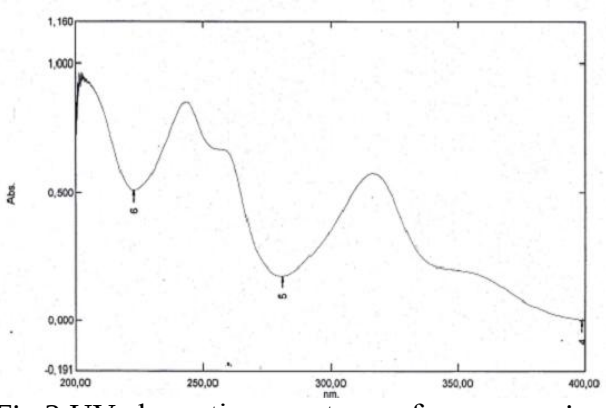



Fig.2 UV absorption spectrum of α-mangostin at a concentration of 10 $\mu$g/ml in methanol (λ max 243 nm; A:0,854)

## 3. Result and Discussion

A UV spectrum of α-mangostin solution in methanolshowed absorption peaks at 243,3 nm and 315,85 nm (Figure 2).The wavelength at 243,3 nm was used for allmeasurements due to no interference from solventabsorbance at 243,3 nm.

*Validation of analytical methods*

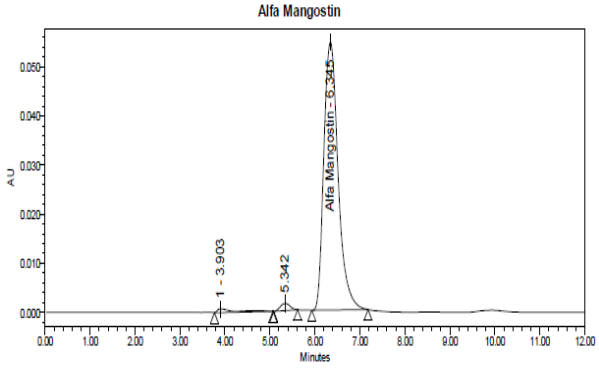
*Specificity*

The chromatograms showed the same retention time between α-mangostin standard solution and a solution of ethyl acetate extract of mangosteen rind were 6.35 minutes and 6.31 minutes for each solution (Figure 3). While methanol as the blank solution did not show the same retention time with these two solutions was 3.89 minutes. So this method was specific to α-mangostin and methanol as the solvent did not give interference in this test.

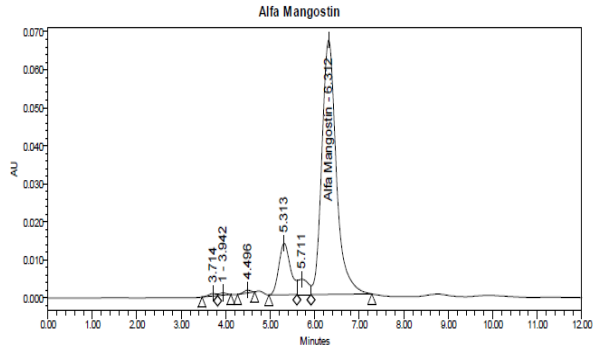
*Linearity and calibration curve*

Linearity of the method was confirmed by preparing standardcurves for the analytical range of 1.0-30.0 µg/mL for determinationof α-mangostin. The equation for the resultant calibrationcurve was y = -8961,29 + 117310,92x; it showed a good correlationbetween analyte peak area and

concentration of the α-mangostinon the analytical range with a linear regression coefficient of 0.9999.The results of LOD and LOQ were found to be 1.19 and 3.98 µg/mL, respectively, which indicating the better sensitivity of this proposed analytical method (Table 1).




(A)




(B)

Fig.3.HPLC chromatograms of α-mangostin standard solution (10.63 µg/mL) (A), and mangosteen peel extract sample solution (contained α-mangostin 13.32 µg/mL) (B).

Table 1. The Method Validation Parameters for Quantification of α-Mangostin by The Proposed HPLC Method

| Parameters | Results |
|---|---|
| Linear range (µg/ml) | 1 – 30 |
| Regression equation* | Y = 117311.X – 8961.3 |
| Correlation coefficient | 0.9999 |
| LOD (µg/ml) | 1.19 |
| LOQ (µg/ml) | 3.98 |

* x is the concentration of α-mangostin in µg/ml
Y isthe peak area at 243,3 nm

Table 2. The Intra-day Precision and Accuracy of The Method for Determination of α-Mangostin

| Intra- day (n=3) | | | |
|---|---|---|---|
| CA (µg/ml) | CF (mean±SD) | RSD (%) | Accuracy (%) |
| 5.32 | 5.32 ± 0.00 | 0.07 | 99.93 |
| 10.63 | 10.66 ±0.01 | 0.12 | 100.32 |
| 15.95 | 16.12 ±0.02 | 0.10 | 101.05 |

* Mean of triplicate analyses in a day.

*Accuracy and precision*

The intra-day and inter-day precision was used to study the variability of the method. Themethod also displayed acceptableprecision, with the RSD values lower than 2%.The inter-day and intra-day precisions of α-mangostin are presented in Table 2 and Table 3. All these data indicated good precision and accuracy. The results showed acceptable precision of the method.

*Recovery*

The recovery at 3 different levels of α-mangostin was 101.28, 101.87, and 100.41 %, with an average of 101.19 % (Table 4). These values indicate good recovery of the method.

*Determination of α-mangostin content in the extracts of G. mangostana rind*

HPLC method with isocratic elution was developed for the determination of α-mangostin in *G. mangostana* rind extracts. The mixture of 0.1%$^{v}/_{v}$ ortho phosphoric acid and acetonitrile (20:80) gaveoptimum chromatographic separation of α-mangostin with the other peaks in the extract (Figure 3 B). The wavelength at 243,3 nm was used for all measurements due to its maximum absorption. The percentage of α-mangostin in the extract was calculated based on the peak area using its calibration curve. The content of α-mangostin in the extract was expressed as

gram per 100 grams of the extract. Each determination was carried out in triplicate.

*Degradation assay*

HPLC studies under different stress conditions indicated the following degradation behaviors. It was found that α-mangostinwas unstable under heat, and oxidative conditions. Nevertheless, the α-mangostin demonstrated decomposition in thermolysis and oxidative conditions, but thedegradation products (DP) have nointerference with this analytical method, as shown in Fig. 4.

In this study, α-mangostin contents of the mangosteen rind extract progressively decreased ($p<0.05$) with thermal and oxidation reaction (Table 5). Possible degradation pathways of the phenoliccompounds may be related to their oxidation,hydrolysis or isomerization [15], leading to the decreased α-mangostin contents in the mangosteen rind extract.

Table 3. The Inter-day Precision and Accuracy of The Method for Determination of α-Mangostin

| CA (µg/ml) | CF (mean ± SD) | RSD (%) | Accuracy (%) |
|---|---|---|---|
| 5.32 | 5.26±0.04 | 0.78 | 98.96 |
| 10.63 | 10.67±0.02 | 0.21 | 103.08 |
| 15.95 | 16.31± 0.16 | 1.01 | 102.23 |

Table 4. Recovery Studies of α-Mangostin in Mangosteen Rind Extract Sample

| Spike Level (µg/ml) | *Mean Recovery (%) |
|---|---|
| 5.32 | 101.28 ± 0.47 |
| 10.63 | 101.87 ± 0.47 |
| 15.95 | 100.41 ± 0.16 |

*The results are mean ± SD of 3 experiments

Table 5. The α-Mangostin Content in *G. mangostana* Fruit Rind Extracts.

| Extract of ethyl acetate | *α-mangostin (% $^{w}/_{w}$) | |
|---|---|---|
| | After thermolysis | After oxidative stress |
| 51.09±0.22 | 44.04±0.40 | 48.06±0.11 |

*The results are mean ± SD of 5 experiments

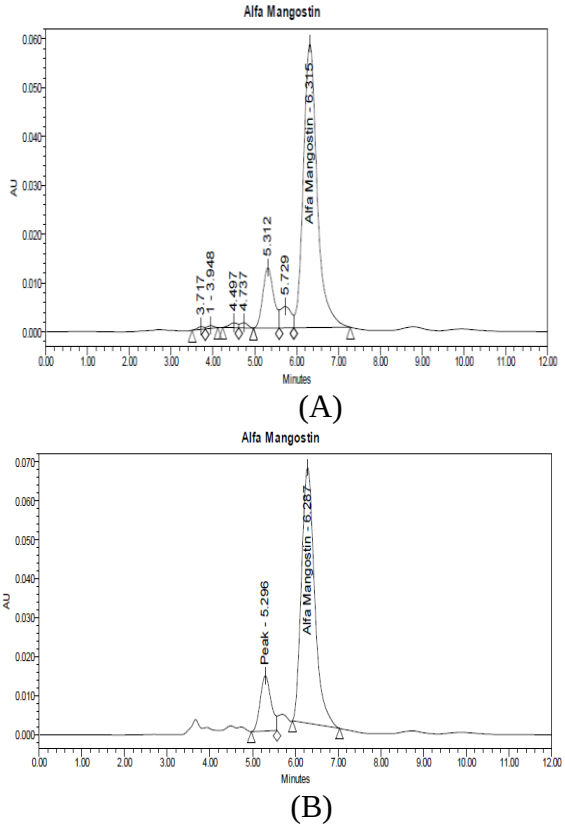



Fig. 4: It shows the typical HPLC chromatograms of α-mangostin understress condition: thermolysis (A) and oxidation with 3% $H_2O_2$ (B)

## Conclusion

The proposed HPLC method promoted high precision, sensitivity and accuracy for quality control of extract of *G. mangostana* L. rind. This proposed method will be useful for quantitative analysis in standardization and quality assessment of extract of *G. mangostana* L. fruit rind for pharmaceutical uses. Alpha mangostin in the ethyl acetate extract of mangosteen rind unstable if treated with termolysis and oxidative reaction.

Whereas contents of α-mangostin in the extract reduced as well as new compound was formed with the retention time of 4.74 minutes for thermolysis reaction.

## Acknowledgements

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## References

[1] Fu, C., A.E.K. Loo, P.P. Chia and D. Huang, "Oligomeric proanthocyanidins from mangosteen pericarps", *J. Agric. Food Chem*., 55: 7689-7694, 2007.

[2] Chin, Y.W., H.A. Jung, H. Chai, W.J. Keller and A.D. Kinghorn, "Xanthones with quinone reductaseinducing activity from the fruits of *Garcinia mangostana* (Mangosteen)", *Phytochemistry*, 69: 754-758, 2008.

[3] Maisuthisakul, P., M. Suttajit and R. Pongsawatmanit, "Assessment of phenolic content and free radical scavenging capacity of some Thai indigenous plants", *Food Chem*.,100: 1409-1418, 2007.

[4] Obolskiy, D.; Pischel, I.; Siriwatanametanon, N.; Heinrich, M., "*Garcinia mangostana* L.: A phytochemical and pharmacological review", *Phytother. Res*.,23, 1047–1065, 2009.

[5] Walker, E.B., "HPLC analysis of selected xanthones in mangosteen fruit", *J. Sep. Sci*., 30, 1229–1234, 2007.

[6] Aisha AFA, Abu-Salah KM, Siddiqui MJ, Ismail Z, and Majid AMSA, "Quantification of α-, β- and γ-mangostin in *Garcinia mangostana* fruit rind extracts by a reverse phase high performance liquid chromatography", *J. Med. Plants Res.*, 6(29): 4526-4534, 2012a.

[7] Pedraza-ChaverrÃ J, Reyes-Fermín LM, Nolasco-Amaya EG, Orozco- Ibarra M, Medina-Campos ON, et al., "ROS scavenging capacity and neuroprotective effect of alpha-mangostin against 3-nitropropionic acid in cerebellar granule neurons", *Exp Toxicol Pathol*, 61: 491-501, 2009.

[8] Jung HA, Su BN, Keller WJ, Mehta RG, and Kinghorn AD., "Antioxidant xanthones from the pericarp of *Garcinia mangostana* (Mangosteen)", *J Agric Food Chem.*, 54(6): 2077-2082, 2006.

[9] Morkini, A., Oussi, D. and Esplugas,S., Oxidation of Aromatic Compounds withUV Radiation/Ozone/Hydrogen Peroxide, http ://www.photon. qulub.es/research/Morkini-cutec-97.doc., 1998.

[10] Pothitirat, W. and W. Gritsanapan, "Thin layer chromatography densitometric analysis of α-mangostin content in *Garcinia mangostana*fruit rind extracts", *J. AOAC Int*., 91: 1145-1148, 2008a.

[11] Pothitirat, W. and W. Gritsanapan, "Quantitative analysis of total mangostins in *Garcinia mangostana* fruit rind", *J. Health Res*., 22: 161-166, 2008b.

[12] Pothitirat W, and Gritsanapan W., "HPLC quantitative analysis method for the determination of α-mangostin in mangosteen fruit rind extract", *Thai Journal of Agricultural Science*, 42(1): 7-12, *2009.*

[13] Andayani R., Wahyuni F.S., Wirasti Y., Dachriyanus, "Development and validation of RP-HPLC method for quantitative estimation of α-mangostin in the rind extract and fractions of *Garcinia mangostana* L. and their cytotoxic activity on T47D breast cancer cell line", *Int J Pharm Pharm Sci.*,Vol 7, Issue 2, 174-178, 2015.

[14] ICH (2005). *Validation Of Analytical Procedures: Text and Methodology Q2(R1).* Available : http://www.ich.org. [accessed 12.08.

[15] Chang, Q., Zuo, Z., Chow, M. S. S. and Ho, W. K. K., "Effect of storage temperature on phenolics stability in hawthorn (*Crataegus pinnatifida* var. major) fruits and hawthorn drink", *Food Chemistry.*, 98: 426-430, 2006.

# The effectiveness test of herbicides 2,4 D, glyphosate, paraquat on low dose as growth regulator on papaya seedling

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## Abstract

In general, herbicide function is the weed controller, but in low dose, it can be useful as a plant growth regulator. The purpose of this study was to determine the effectiveness of the herbicide 2,4 D, Glyphosate and Paraquat in low dose as a growth regulator on Papaya Seedlings. The experimental design was a randomized block design (RCBD) with two factors. The first factor is herbicide type, consisting of four types ie: control; 2.4 D; Glyphosate; and Paraquat. The second factor is herbicide dose, consisting of three levels, ie: 2 mg/l; 0.2 mg L-1; and 0.02 mg L-1. Each treatment was repeated three times. The result showed that the herbicide 2,4 D on a low dose of 0.02 mg/l can increase the plant growth, but when the dose was increased up to 2 mg/l, plants began to show poisoning symptoms. The same condition occured on glyphosate and paraquat, although the dose level still can be used by plants.

Keywords: herbicide; 2,4 D, glyphosate, paraquat

## 1. Introduction

Herbicides are chemicals compound which can be used as the weed controller on plant cultivation. Herbicide residues are usually discharged into drainage canal that can pollute the environment and damage the ecosystem [1]. Herbicides can stop the growth of weeds temporarily or permanently depending on the dose used. In the other words, the type and toxicity level of the chemical compound on the herbicide determine the meaning of the herbicide [2]. Herbicides can control weeds before bothering the crop cultivation, it also can inhibit the damage of crop cultivation root, more effective to kill the perennial weeds and in low doses can have a function as a growth hormone [3].

Plant growth regulators have an important role to control biological processes in plant tissue. Its important role is regulating the growth speed from each plant tissue to form whole plants. Activities of growth regulators in plants depend on the type, chemical structure, concentration, genotype, and phases of plant physiology [4]; [5].

The research on the use of herbicides as the plant growth regulator has been widely performed. 2,4 D in low dose (0.02 to 0.2 mgl-1) and glyphosate (0.02 mgl-1) are able to stimulate the algaegrowth [6]. 2,4 D is also capable tostimulate the growth of plant cells. Glyphosate can increase the growth of maize, soybean, eucalyptus and pine [7]. [8]Paraquat is one of the chemicalcompound that can induce the resistance in tobacco plants. [9] The use of paraquat at low dose (108 g / ha) can increase the taproot biomass in carrot. [10] Herbicide application can stimulate the root formation in Chinese Tallowtree (Triadicasebifera).

Besides useful as the growth hormones, herbicides are also used by plant breeders to chromosome doubling. Herbicides which often used are herbiside which has an active ingredient oryzalin and trifluralin. This

material in very low dose can inhibit mitotic division, so it caused to induce the chromosome doubling. Both types of this herbicides is more effective and cheaper than colchicine, a chemical compound that previously used for the duplication of chromosomes [11];[12]. Therefore, one of the reasons the use of herbicides as the growth hormone in this study is due to herbicide easily available and affordable.

The objective of this research was to examine the effectiveness of the herbicide 2,4 D, glyphosate, and paraquat at the low dose as a growth regulator on papaya seedlings.

## 2. MaterialandMethods

The research was conducted from February to May 2015 in the Experimental Garden Aripan, Indonesian Tropical Fruit Research Institute (ITFRI) at an elevation of ± 345 m above sea level. The plant material used are papaya seeds varieties Dampit, manure, herbicides with the active ingredient 2,4 D, glyphosate and paraquat. The tools used include seed beds, polybag size of 15 cm x 30 cm, buckets, hoes, machetes, hand sprayer, vernier caliper, labels, scales and stationery.

The experiment was arranged in a complete randomized block design (RCBD) with two factors. Each treatment consists of two groups. The first factor is the type of herbicide (A), consisting of four levels ie water as a control (A0); 2.4 D (A1); glyphosate (A2) and paraquat (A3). The second factor is the dose of herbicide (B), consisting of three levels ie 2 mgl-1 (B1), 0.2 mg L-1 (B2); and 0.02 mg L-1 (B3). The whole experiment consisted of 36 experimental units and each experimental unit consisted of 10 plants.

Papaya seeds soaked for one night, then it sown in moist tissue paper for 7-10 days. Sprouts planted in polybags with media, a mixture of soil and manure in the ratio 1: 1. The treatment given to plants by sprayed using a sprayer every week, and beginning at age 2 Weeks After Planting (WAP) until the plant aged 6 WAP. Observations were made up to 7 WAP.

The variables observed were plant height, stem diameter, number of leaves, leaf bone length, leaf width and root length. The data were analyzed on analysis of variance. If the value of F count is larger than F table 5% followed by Duncan's Multiple Range Test (DMRT) at the 5% significance level.

## 3. Result andDiscussion

*3.1.Plant Height*

Analysis of variance to the height of the plants at low doses of herbicide treatments showed no significant interaction (Table 1). The treatment used does not affect to increase the plant height in papaya seedlings (Table 1). Allegedly, all treatments provide similar abilityinincreasing the plant height. The highest height of the average crop height obtained on the application of glyphosate 2 mgl-1, ie 16.07 cm, and reduction of 2.4 D dose became 0.02 mgl-1 was also able to increasethe plant height became 16.06 cm. [13] Herbicides which is similar to auxin such as dicamba and 2,4-D are a cheap growth regulator. These compounds are used to control fruit maturity, induction of somatic embryogenesis, and increase the fruit size. [14], the effectiveness of herbicide applications is determined by herbicide dose. If the dosewas too high, it can damage and even kill plants which cultivated.

Table 1. Papaya PlantHeight at Age 7 WAP

| Herbicide ingredients | Doses of herbicide | | |
|---|---|---|---|
| | 2 mg/l | 0,2 mg/l | 0,02 mg/l |
| Water | 12,70 | 14,75 | 15,61 |
| 2,4 D | 13,28 | 15,82 | 16,06 |
| Glyphosate | 16,07 | 15,87 | 15,72 |
| Paraquat | 14,87 | 14,33 | 11,84 |

The numbers in rows and columns not significant by F test at 5%

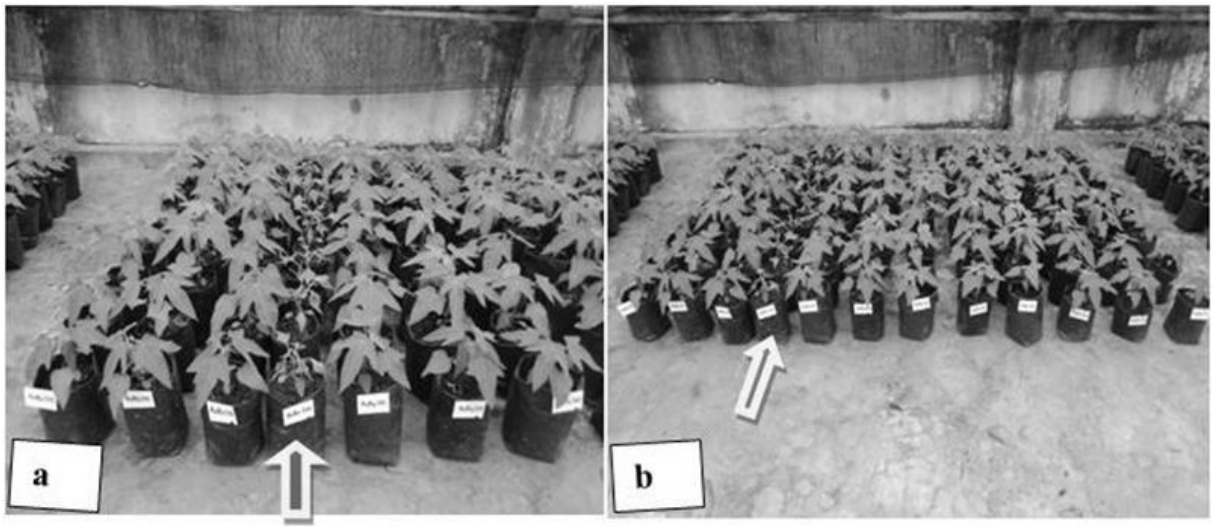


Fig. 1. Shows that the application of 2.4 D at a dose 2 mgl-1 has begun to inhibit the growth of papaya seedlings with the experiencing of rolling leaves, thus slowing down the leaf drop. [15] The symptoms of plant poisoning caused by the herbicide 2,4-D include the epinasty, formation of tumor tissue, the curved stem and easily broken, and leaf rollig.

Table 2. Papaya Leaves Number on 7 WAP

| Herbicides ingredient | Doses of herbicide | | |
|---|---|---|---|
| | 2 mg/l | 0,2 mg/l | 0,02 mg/l |
| Water | 9,07 a B | 9,07 a AB | 9,38 a AB |
| 2,4 D | 11,01 a A | 9,73 b A | 9,08 b AB |
| Glyphosate | 8,89 a B | 9,20 a AB | 9,57 a A |
| Paraquat | 9,13 a B | 8,94 a B | 8,57 a B |

The numbers on the line followed by the same lowercase letters and numbers in a column followed by the same capitalization respectively not significant according to DMRT level of 5%

Tabel 3. Papaya LeafLength on 7 WAP

| Herbicides ingredient | Doses of herbicide | | |
|---|---|---|---|
| | 2 mg/l | 0,2 mg/l | 0,02 mg/l |
| Water | 9,94 a A | 10,20 a AB | 10,75 a A |
| 2,4 D | 6,20 b B | 9,06 a B | 10,02 a A |
| Glyphosate | 10,25 a A | 10,19 a A | 10,48 a A |
| Paraquat | 10,31 a A | 10,58 a A | 9,60 a A |

The numbers on the line followed by the same lowercase letters and numbers in a column followed by the same capitalization respectively not significant according to the DMRT level of 5%.

*3.2. Leaves Number*

Analysis of variance on the number of leaves shows significance interactions (Table 2). The application of 2,4-D at a dose 2mgl$^{-1}$ shows the average of the highest of leaves number is 11.01 leaves(Table 2). This treatment is significantly differen if the dose decreased to be 0,2 mgl$^{-1}$and 0,02 mgl-1.It also showed a significantly different effect on control, glyphosate and paraquat with the same dose, 2 mgl$^{-1}$. The high of leaves number at age 7 MST does not indicate the good growth, due to the application of 2.4 D at a dose 2 mgl$^{-1}$ showed poisoning symptoms (Fig. 1. (a), (b).

*3.3. Length of leaves*

Analysis of variance trough the leaf length shows interaction of significantly different (Table 3). The application of 2.4 D at a dose 2 mgl-1 shows the average length of the shortest leaf is 6.20 cm, which significantly different from the application of a dose 0.2 mgl-1 and 0.02 mgl-1 (Table 3). 2.4 D treatment with a dose 2 mgl-1 also shows a significantly different effect with controls, glyphosate and paraquat at the same

dose, ie 2 mgl-1. The best of leaf lenght obtained on the control, followed by paraquattreatment at a dose 0.2 mgl-1 with a leaf length of 10.75 cm and 10.58 cm. From the table shows that 2.4 D at a dose 2 mgl-1 has begun to inhibit the growth of papaya seedlings, so that the process of leaf growth becomes stunted. This is in accordance with the opinion ofthat 2.4 D at a dose 20 mgl-1 has begun to inhibit algae growth significantly, and at a dose 2 mgl-1can reduce the yield of photosynthesis about 80% compared to controls [1].

*3.4.Leaf width*

ANOVA results at the variable of leaves width also showa significant interaction effect (Table 4). 2.4 D Treatment with a dose 2mgL-1 shows the average of the lowest leaf width is 7.49 cm. These results are significantly different to the treatment of 0.2 mgl-1 and 0.02 mgl-1, where a reduction in the dose of 2.4 D is able to increase the width of the leaves of plants. According to [16], herbiside 2.4 D gives different effects on plant growth depending on the dose given. At very low dose can stimulate the growth of legumes.

2.4 D treatment with a dose 2 mgl-1 was not significantly different to controls, but significantly different with the application of glyphosate and paraquat at a dose 2 mgl-1. Applications 2.4 D at a dose 2 mgl-1 stunted the leaf growth process. [17], stated that the herbicide 2,4 D in high dose can disrupt plant growth, and can cause death, especially in the group of dicotyledonous plants. The initial symptoms shown by the emergence of chlorosis [18].

*3.5.Stem diameter*

Analysis of variance of the stem diameter shows no significant interaction (Table 5). Table 5 shows that stem diameter obtained ranged from 3.53 cm to 6.45 cm. The highest average diameter obtained on application 2.4 D at a dose 0.02 mg/l. It is seen that at low doses of 2.4 D is effective in increasing cell division, in line with [2], at low dose 2.4 D can encourage cell division, the growth of plants and improve the germination of seeds.

*3.6.Root length*

Analysis of variance of the variables root length also shows no significant interaction (Table 6).

Table 4. PapayaWidth of Leaves on 7 WAP

| Herbicides ingredient | Doses of herbicide | | |
|---|---|---|---|
| | 2 mg/l | 0,2 mg/l | 0,02 mg/l |
| Water | 10,13 b A | 10,89 ab A | 11,75 a A |
| 2,4 D | 7,49 b B | 9,75 a A | 10,76 a AB |
| Glyphosate | 10,92 a A | 10,78 a A | 11,17 a AB |
| Paraquat | 10,80 a A | 11,03 a A | 10,00 a B |

The numbers on the line followed by the same lowercase letters and numbers in a column followed by the same capitalization respectively not significant according to the DMRT level of 5%.

Table 5.PapayaStem Diameter on 7 WAP

| Herbicide ingredients | Doses of herbicide | | |
|---|---|---|---|
| | 2 mg/l | 0,2 mg/l | 0,02 mg/l |
| Water | 4,56 | 4,74 | 5,03 |
| 2,4 D | 3,53 | 4,83 | 6,45 |
| Glyphosate | 4,66 | 4,80 | 4,95 |
| Paraquat | 5,04 | 4,64 | 4,79 |

The numbers in rows and columns not significant by F test at 5%

Table 6. Papaya Length of Roots on 7 WAP

| Herbicides ingredient | Doses of herbicide | | |
|---|---|---|---|
| | 2 mg/l | 0,2 mg/l | 0,02 mg/l |
| Water | 17,83 | 19,70 | 21,59 |
| 2,4 D | 18,79 | 21,83 | 20,58 |
| Glyphosate | 21,40 | 23,15 | 21,63 |
| Paraquat | 21,15 | 20,87 | 17,17 |

The numbers in rows and columns not significant by F test at 5%

Table 7. Comparison of the Effectiveness of 2.4 D, Glyphosate and Paraquat as a Growth Regulator

| Variables | Dosisherbisida (mg/l) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2,4 D | | | Glyphosate | | | Paraquat | | |
| | 2 | 0,2 | 0,02 | 2 | 0,2 | 0,02 | 2 | 0,2 | 0,02 |
| Plant height | - | - | - | - | - | - | - | - | - |
| Leaves number | √ | - | - | - | - | - | - | - | - |
| Leaf length | √ | - | - | - | - | - | - | - | - |
| Leaf width | √ | - | - | - | - | - | - | - | - |
| Stem diameter | - | - | - | - | - | - | - | - | - |
| Roots length | - | - | - | - | - | - | - | - | - |

Note :√ (significant) - (not significant)

The average length of the longest root obtained on glyphosate aplication with a dose 0.2 mg/l, followed by 2.4 D aplication at a dose 0.2 mg/l with a length of 23.15 cm and 21.83 cm. Table 6 can explain that the dosage and type of herbicide have not been able to increase the growth of root length on papaya seedlings.

Generally, the herbicide 2,4 D shows a significant influence on the growth of papaya seedlings. According to [19] in several types of plants, 2,4-D application at 1.20 ppm can generate the increasing of plant height, length of roots, wet weight and dry weight of plants.

The herbicide can essentially be used as plant growth regulators (Table 7). Glyphosate and paraquat can be used at a dose 0.02 mgl-1 and 2 mgl-1. While herbicide 2,4 D can only be used as a plant growth regulator until the doses 0.02 mgl-1.

## Conclusion

Testing the effectiveness of herbicide 2,4 D, Glyphosate, and Paraquat at low doses give different responses to plants. 2,4 D application on a low dose 0.02 mg/l can promote plant growth, increasing dose causes plants showing poisoning symptoms.The same conditioan also applies to glyphosate and paraquat, although the dose given are still in the stage that can be used by plants, Dosage of Glyphosate applications, and Paraquatare 0,02 mg/l to 2 mg/l and 2.4 D $\leq$ 0.2 mg/l. Thesedoseare recommended dose for the use of herbicides as a plant growth regulator.

## Acknowledgements

"Pleaseprovideyouracknowledgement here."

## References

[27] Wong, PK. Effects of2,4 D, glyphosate and paraquat on growth, photosyntesis and chlorophyll-a synthesis of Scenedesmus quadricauda berb 614. Chemosphere 41 (2000) 177-182, 2000.

[28] Moenandir, J. PengantarIlmuPengendalianGulma. Rajawali Press. Jakarta. 1990.

[29] Chairul, M. S, Mulyadi, Idawati. Translokasi herbisida 2,4 D-$^{14}$C pada tanaman gulma dan padi pada system persawahan. Risalah pertemuan ilmiah penelitian dan pengembangan teknologi isotop dan Radiasi 2000. Hal 151-155. 2000.

[30] Satyavathi, V.V., P.P. Jauhar, E.M. Elias, and M.B. Rao. Genomics, molecular genetic and biotechnology efects of growth regulators on in vitro plant regeneration. Crop Sci. 44:1839-1846. 2004.

[31] Lestari, EG. Peranan zat pengatur tumbuh dalam perbanyakan tanaman melalui kultur jaringan. Jurnal Agro Biogen 7(1):63-68. 2011.

[32] Draber, W. Plant growth regulators. In: Buchel, K. H. Chemistry of pesticides. Wiley, New York, pp. 393-405.1983.

[33] Velini, ED, E. Alves, MC. Godoy, DK Meschede, RT. Souza and SO. Duke. Glyphosate applied at low doses can stimulate plant growth. Pest ManagSci 64:489–496, 2009.

[34] Edreva, A. A novel strategy for plant protection induced resistance. Journal of cell and Molecular biology. 3 : 61-69. 2004.
[35] Cochavi, A., Guy Achdari, Evgeny Smirnov, Baruch Rubin, and HananEizenberg. Egyptian Broomrape (Phelipancheaegyptiaca) Management in Carrot under Field Conditions. Weed Technology. 29:519–528, 2015.
[36] Enloe, SF., Nancy J. Loewenstein, Douglas Streett, and Dwight K. Lauer. Herbicide Treatment and Application Method Influence Root Sprouting in Chinese Tallowtree (*Triadicasebifera*). Invasive Plant Science and Management 2015 8:160–168, 2015.
[37] Ascough, GD and J. Van Staden.Effectiveness of Colchicine and Oryzalin at Inducing Polyploidy in *Watsonialepida* N.E. Brown.*Hortscience* 43(7): 2248–2251, 2008.
[38] Viehmannova, I,´ EF. Cusimamani, M.Bechyne, M. Vyvadilova, M Greplova. In vitro induction of polyploidy in yacon (*Smallanthussonchifolius*). Plant Cell Tiss Organ Cult 97:21–25, 2009.
[39] Velini, ED, M.L.B. Trindade, L.R.M. Barberis, and S.O. Duke. Growth Regulation and Other Secondary Effects of Herbicides. Weed Science 58:351–354, 2010
[40] Ashton dan Craft. Mode of Action of Herbicides. John Willey and Son, New York. 1981.
[41] Grossmann K.Mediation of herbicide effects by hormone interactions. *Journal of Plant Growth Regulation* 22, 109–122, 2003.
[42] Pazmiño, D.M.,M.R. Serrano, M.C.R. Puertas, A.A Ruiz, L.A. delRío and L.M Sandalio. Differential response of young and adult leaves to herbicide 2,4-dichlorophenoxyacetic acid in pea plants: role of reactive oxygen species.Plant, Cell and Environment 34, 1874–1889. 2011.
[43] Grossmann, K.Auxin herbicides: current status of mechanism and mode of action. Pest Manag Sci. 66: 113–120, 2009.
[44] Grossmann K.Mediation of herbicide effects by hormone interactions. *Journal of Plant Growth Regulation* 22, 109–122, 2003.
[45] Podesta, F., U. Kalsum, dan E. Mareza.Kajian Konsentrasi Zat Pengatur Tumbuh 2,4-D Terhadap Viabilitas, Vigor dan Pertumbuhan Benih pada Beberapa Genotipe Tanaman Jarak Pagar (*Jatrophacurcas* Linn.). *Jurnal Akta Agrosia,* 11 (1) : 19, 2008.
[46] A. Karnik, "Performance of TCP congestion control with rate feedback: TCP/ABR and rate adaptive TCP/IP," M. Eng. thesis, Indian Institute of Science, Bangalore, India, Jan. 1999.
[47] J. Padhye, V. Firoiu, and D. Towsley, "A stochastic model of TCP Reno congestion avoidance and control," Univ. of Massachusetts, Amherst, MA, CMPSCI Tech. Rep. 99-02, 1999.
[48] *Wireless LAN Medium Access Control (MAC) and Physical Layer (PHY) Specification*, IEEE Std. 802.11, 1997.