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
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The effect of fermentation duration and level of *aspergillus niger* on nutrient quality of pineapple leaves

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Abstract. This study aimed to determine the nutritional quality of fermented pineapple leaves fermented at different duration using different levels of *Aspergillus niger* mold. The data obtained were analyzed using analysis of variance (ANOVA) with significant level of 5% and or 1% and continued with the Least Significant Difference (LSD) test. This study used an experimental method using a factorial design with Completely Randomized Design (CRD) consisting of 3 x 3 treatments, each treatment consisted of 3 replications so that there were 27 experimental units. The first factor was addition of *Aspergillus niger* levels of 0%, 2%, and 4% in a substrate, while the second factor was fermentation duration of 0 days, 6 days, and 12 days. The result showed that there was no interactions between fermentation duration and the level of *Aspergillus niger* on dry matter, organic matter, and crude protein of fermented substrate, but interactions occurred to ether extract and crude fiber content. Combination of treatment of *Aspergillus niger* 4% and fermentation duration of 0 day was the best in this research in producing the lowest crude fiber content of the fermented product.

INTRODUCTION

Feedstuff is one of the factors that play a major role in livestock productivity and holds the largest financing in a livestock business. The cost of feedstuff can reach the range of 60-70% of the total production cost [1]. The usual feed given to ruminants is forage. However, in some areas in Indonesia, the amount of available forage cannot meet the feed needs of ruminants. Factors that affect the availability of forage feeds include the availability of raw materials, climate, season, soil conditions, and land availability [2]. Therefore, agricultural and agro-industrial waste can be used as an alternative feed to overcome the shortage of forage as ruminant animal feed.

One of the agricultural and agro-industrial wastes is pineapple waste. Indonesia is one of the well-known commercial fruit producing countries in Southeast Asia. One type of fruit that is cultivated in Indonesia is pineapple. One of the areas in Indonesia that cultivates pineapple plants is Lampung Province. Persero Limited Great Giant Foods is a company that focuses on producing processed pineapple in Lampung Province. PT. Great Giant Foods is located at Jalan Lintas Sumatra KM.77, Terbanggi Besar District, Central Lampung Regency, Lampung Province. PT. Great Giant Foods has a plantation area of \pm 80,000 hectares with the pineapple variety grown is Smooth cayenne [3].

Pineapple is a horticultural commodity that is always available throughout the year because planting and harvesting pineapples do not depend on the season. Pineapple has the scientific name *Ananas comosus* (L.) Merr and belongs to the Bromeliaceae family. Pineapple can grow and bear fruit in the highlands up to 1,000 meters above sea level. The origin of this pineapple plant is from mainland South America then spread throughout the tropical world, including Indonesia. At the time of harvesting, pineapple plants produce very abundant waste so that it can potentially be a source of ruminant feed. Pineapple leaves are one of the wastes from pineapple plants which are abundant and available sustainably in the long term. Based on previous researchers, pineapple leaves are pineapple plant residues which have the highest percentage value, which is about 90% of the total waste generated from pineapple plants. In

terms of nutrition, pineapple leaves contain 85% water, 15% dry matter, 97.79% organic matter, 9.1% crude protein, 23.6% crude fiber, 1.6% ether extract [4]. Based on these nutritional content, pineapple leaves can be used as an alternative feed for ruminants when forage supplies are limited, although the crude fiber content is still high and the crude protein content is low. Therefore, it is necessary to know the quality of the nutritional content of pineapple leaves. Optimizing the nutritional content of pineapple leaves can be done by fermentation. From this explanation, this study was conducted to determine the effect of fermentation duration and the level of *Aspergillus niger* on the nutrient content of pineapple leaves.

MATERIALS AND METHODS

This research activity was carried out from January 2022 to March 2022, at the Animal Feed Laboratory, Department of Animal Husbandry, Faculty of Agriculture, University of Lampung. This research was carried out in three stages, namely the first stage of *Aspergillus niger* culture propagation, sample preparation, and lastly analysis of nutrient content.

ASPERGILLUS NIGER CULTURE PROPAGATION

Culture propagation of *Aspergillus niger* was done as following procedures.

- a) washing rice;
- b) adding water as much as 400 cc/kg of rice;
- c) cooking rice until half cooked;
- d) steaming half-cooked rice for 30 minutes then let it cool;
- e) mixing it evenly as much as 3 petri/kg rice;
- f) incubating for 5 days covered with plastic wrap that has been perforated;
- g) drying in an oven at 40°C for 5 days;
- h) refining the culture results and are ready to be used in feed fermentation [5]

Pineapple leaf preparation for fermentation

Pineapple leaves were taken directly from PT Great Giant Foods which is located in Terbanggi Besar District, Central Lampung Regency. The leaves taken include leaves that are still good and not old. Pineapple leaves are cut into 1 cm long, then sterilized by means of steaming using a pot for 25 minutes and then allowed to stand until cold.

The stages of pineapple leaf fermentation as following.

- a) cut the pineapple leaves with a size of 1 cm, then weigh the pineapple leaves;
- b) steam pineapple leaves using a steamer for 25 minutes, then cooled and weighed;
- c) put pineapple leaves into a bowl and add *Aspergillus niger* 0g, 80g, and 160g;
- d) stir in the basin until evenly distributed, then put the pineapple leaves into a plastic that has been perforated and tied with raffia rope and marked according to the treatment
- e) store in a room with a temperature of 28°C and ferment for 0 days, 6 days and 12 days.

Data analysis

Fermented substrates were analyzed using the proximate method [6] for its nutrient content (dry matter, organic matter, crude protein, ether extract, and crude fiber). The nutrient content data that has been obtained were analyzed using Analysis of Variance (ANOVA) at the level of 5% and or 1%. If the results of the analysis of variance show a significant effect, then the analysis was continued with the Least Significant Difference (LSD) test. This study used an experimental method using factorial design with completely randomized design (CRD) consisting of 3 x 3 treatments, each treatment consisted of 3 replications so that there were 27 experimental units. The first factor was fermentation duration (0, 6, and 12 days), while the second factor was the level of *Aspergillus niger* in a substrate (0, 2, and 4%).

RESULTS AND DISCUSSION

The nutrient content (dry matter, organic matter, crude protein, ether extract and crude fiber) of fermented pineapple leaves using different level of *Aspergillus niger* and different duration of fermentation are presented in Table 1.

TABLE 1. Nutrient content of fermented pineapple leaves with different level of *Aspergillus niger* 0% (L0), 2% (L1) and 4% (L2) in a substrate and different duration of fermentation for 0 day (D0), 6 days (D1), and 12 days (D2).

Treatment combination	Nutrient content (%)				
	DM	OM	CP	EE	CF
<i>Simple effect</i>					
L0D0	13,40	92,16	7,34	1,09 ^d	22,17 ^{cd}
L0D1	10,10	89,25	10,37	1,70 ^{cd}	25,53 ^a
L0D2	7,48	89,37	8,88	2,58 ^{ab}	24,93 ^{ab}
L1D0	13,44	90,18	8,72	2,18 ^{abc}	23,33 ^{bc}
L1D1	9,20	89,77	10,67	2,79 ^a	22,52 ^{cd}
L1D2	7,35	85,74	9,38	2,54 ^{ab}	26,11 ^a
L2D0	14,61	92,64	7,55	1,91 ^{bc}	17,42 ^e
L2D1	9,78	89,43	10,93	1,73 ^{cd}	21,52 ^d
L2D2	7,74	86,64	9,75	2,04 ^{bc}	20,89 ^d
<i>Main effect</i>					
L0	10.33±2.97	90.26±1.65	8.86±1.52	1.79±0.75	24.21±1.79 ^a
L1	9.99±3.12	88.56±2.45	9.59±0.99	2.50±0.31	23.99±1.88 ^a
L2	10.71±3.53	89.57±3.00	9.41±1.72	1.89±0.16	19.94±2.21 ^b
D0	13.82±0.69 ^a	91.66±1.30 ^a	7.87±0.74 ^c	1.73±0.57	20.97±3.13
D1	9.69±0.46 ^b	89.48±0.26 ^{ab}	10.66±0.28 ^a	2.07±0.62	23.19±2.09
D2	7.52±0.20 ^c	87.25±1.89 ^b	9.34±0.44 ^b	2.39±0.30	23.98±2.74

Note: The mean with different letter superscripts in the same column shows a significant difference ($P < 0.05$). DM (Dry Matter); OM (Organic Matter); CP (Crude Proteins); EE (Ether Extract); CF (Crude Fiber).

Effect of Treatment on Dry Matter of Fermented Pineapple Leaf

Based on the results obtained in the analysis of variance, the treatment of *Aspergillus niger* with levels of 0%, 2%, and 4% gave the results that there was an insignificant effect ($P > 0.05$) on the dry matter content of fermented pineapple leaves, while fermentation duration of 0 days, 6 days, and 12 days showed a very significant effect ($P < 0.01$) on dry matter content of fermented pineapple leaves. The results of the analysis of variance also showed that there was no interaction ($P > 0.05$) between *Aspergillus niger* levels and fermentation durations on dry matter content of fermented pineapple. Furthermore, the addition of different levels of *Aspergillus niger* showed no significant effect on dry matter content of fermented pineapple leaves, however numerically there was a change in dry matter content during the fermentation process. The highest dry matter content (10.71%) was obtained at the 4% level in a substrate and the lowest dry matter content (9.99%) was obtained at the 2% level in a substrate. One of the factors that cause changes in dry matter content is the growth and development of molds during the fermentation process. According to [7], the more the amount of mold added in the fermentation process, the higher the water content that will be produced because the fermentation process will be faster. The higher the water content, the lower the dry matter content of fermented pineapple leaves. [8] also added that the breakdown of carbohydrates by molds followed by the release of energy in the form of heat, CO₂, and H₂O will cause the substrate to lose dry matter components.

Further test of the least Significant Difference (LSD) based on the fermentation duration of pineapple leaves showed that the highest dry matter content was found in the 0-day fermentation (L0) treatment, namely 13.82%, then followed by the 6-day fermentation (L1) of 9.69%, and the lowest value was found in the 12-day fermentation duration (L2) of 7.52%. The results of the LSD test showed that the best results for the dry matter content of fermented pineapple leaves were found in the 0 day (L0) fermentation duration treatment. The decrease in dry matter content

during the fermentation process was caused by the reshuffling of organic matter content in the form of carbohydrates into energy for the growth and activity of *Aspergillus niger*. The reshuffle process will produce by-products in the form of carbon dioxide and water. The by-product in the form of water causes the dry matter content to decrease. According to [9], the longer the fermentation time will result in an increase in the amount of mold growth where the growing mold will contribute most of the water content in the fermentation. According to [10], the decrease in dry matter content occurs due to overhaul by microorganisms (bacteria and fungi) used for growth. [11] also added that in fermentation there will be an increase in the water content in the substrate due to the decomposition of dry matter by bacteria which is used as an energy source for the formation of new cells. Increasing the water content in a fermentation will cause a decrease in the dry matter content. The increase in water content causing a decrease in the dry matter content indicated that during the fermentation process *Aspergillus niger* mold experienced optimal growth.

Effect of Treatment on Organic Matter of Fermented Pineapple Leaf

Based on the results obtained in the analysis of variance, the treatment of *Aspergillus niger* with doses of 0%, 2%, and 4% showed that there was an insignificant effect ($P>0.05$) on the organic matter content of fermented pineapple leaves, while the Fermentation duration of 0 days, 6 days, and 12 days showed a very significant effect ($P<0.01$) on the organic matter content of fermented pineapple leaves. The average value of organic matter content of pineapple leaves with *Aspergillus niger* levels of 0% (L0), 2% (L1), and 4% (L2), were 90.26%, 88.56%, and 89.57%, respectively, while based on the length of fermentation of 0 days (D0), 6 days (D1), and 12 days (D2), the results were 91.66%, 89.48%, and 87.25%, respectively. The results of the analysis of variance also showed that there was no interaction ($P>0.05$) between *Aspergillus niger* levels and fermentation duration on the organic matter content of fermented pineapple leaves. This shows that the treatment of *Aspergillus niger* levels with different fermentation duration did not affect each other on the organic matter content of fermented pineapple leaves. Furthermore, the addition of *Aspergillus niger* levels of 0%, 2%, and 4% showed no significant effect on the organic matter content of fermented pineapple leaves, however, numerically there was a change in organic matter content during the fermentation process. The highest organic matter content was obtained at the 0% level which was 90.26% and the lowest content was obtained at the 2% level which was 88.56%. One of the factors that play a role in the high and low levels of organic matter is microbial activity in the breakdown of substrate content in the fermentation process. [12] stated that microorganisms utilize organic matter from the substrate to be degraded into sugar, protein, starch, hemicellulose and cellulose for growth. This will cause a decrease in the organic matter content of the substrate. Another factor that causes high and low levels of organic matter is the production of enzymes produced from molds. According to [13], the more *Aspergillus niger*, the higher the enzyme production, so that the amount of organic matter that is reorganized is also increasing.

Based on the LSD test, the longer fermentation of pineapple leaves causes a significant decrease in organic matter levels. Decreased levels of organic matter can occur due to chemical changes during the fermentation process due to microbial activity that degrades components of organic matter as a carbon source for development, growth, and activity. This is in accordance with the opinion of 10 which stated that the content of organic matter decreases with increasing incubation time where the longer the incubation time will provide an opportunity for microbes to utilize organic material derived from the substrate to degrade organic matter such as sugar, protein., starch, hemicellulose, and cellulose for its growth. Therefore, the longer the fermentation process, the lower the organic matter content of pineapple leaves.

Effect of Treatment on Crude Protein Content of Fermented Pineapple Leaf

The results showed that there was no interaction between *Aspergillus niger* level and duration of fermentation on crude protein content of fermented pineapple leaves ($P>0.05$). Based on the average fermentation time, the highest crude protein content of pineapple leaves at 6 days (D1), 12 days (D2), and 0 days (D0) were of 10.66%, 9.34%, and 7, 87%, respectively. The average crude protein content with *Aspergillus niger* level of 2% (L1), 4% (L2), and 0% (L0) were 9.59%, 9.41%, and 8.86%, respectively. The results of the analysis of variance showed that the crude protein content in pineapple leaves with the addition of *Aspergillus niger* levels of 0%, 2%, and 4% was not significantly different ($P>0.05$), this was presumably because pineapple leaves had a high crude fiber content, so *Aspergillus niger* cannot develop properly to produce protease enzymes optimally. Fermentation duration treatment had a very significant effect on the crude protein content of pineapple leaves ($P<0.01$). Based on the LSD test, it can be seen that the highest crude protein content was produced in the D1 treatment which was significantly different from the D0 and D2 treatments, while the lowest results was obtained in the D0 treatment. This is in accordance with the results of

Mirnawati (2007) research that fermented palm oil cake using *Aspergillus niger* for 6 days can increase the crude protein content from 18.67% to 27.70%. This is in line with the research results of 14, that fermentation of pineapple waste using *Aspergillus niger* 2% with a fermentation duration of 4 days can increase crude protein content from 4.41% to 9.55%, because it is influenced by the presence of protein donated due to the growth of *Aspergillus niger*. The crude protein content of pineapple leaves fermented for 12 days decreased, this was presumably because *Aspergillus niger* had entered the death phase so that its activity decreased. 13 stated that during the 6-day fermentation period, crude protein levels decreased due to the growth of *Aspergillus niger* which was already in the death phase so that it underwent lysis and the proteins contained in its cells decomposed into non-proteins, for example in the form of ammonia. This is in line with the opinion of [5], that on the 5th and 6th day of incubation, microorganisms experienced a death phase which indicated that *Aspergillus niger* was no longer working optimally. This is because the availability of nutrients is decreasing and running out.

Effect of Treatment on Ether Extract Content of Fermented Pineapple Leaf

Based on analysis of variance, the treatment of *Aspergillus niger* with doses of 0%, 2%, and 4% with a fermentation duration of 0 days, 6 days, and 12 days showed that there was a very significant interaction effect ($P < 0.01$) on ether extract content of fermented pineapple leaves. The average value of ether extract content of pineapple leaves with *Aspergillus niger* levels of 0% (L0), 2% (L1), and 4% (L2) were 1.79%, 2.50%, and 1.89%, respectively, while based on the length of fermentation 0 days (D0), 6 days (D1), and 12 days (D2), the results were 1.73%, 2.08%, and 2.39%, respectively. This shows that the treatment of *Aspergillus niger* levels with different fermentation duration affected each other on the ether extract content of fermented pineapple leaves. The lowest ether extract content in pineapple leaf fermentation was found in 0% *Aspergillus niger* and 0 day fermentation duration, which was 1.09%, while the highest ether extract content was found in L1D1 or 2% *Aspergillus niger* with fermentation duration 6 days, which was 2.79%. The results of the LSD further test showed that the best results for ether extract content of fermented pineapple leaves were found at 0% *Aspergillus niger* level treatment with 0 days of fermentation time (L0D0).

The increase in ether extract content with the increase in *Aspergillus niger* was caused by *Aspergillus niger* growth and was able to convert high-carbohydrate substrates into fat. According to [15], microorganisms utilize 33 organic materials (protein, fat, carbohydrates, and vitamins) from the substrate to be degraded into sugar, protein, starch, hemicellulose and cellulose to produce energy to support their growth. [16] added that the longer the fermentation is in line with the longer the opportunity for the mold to digest cellulose, so that more fat is produced. Therefore, the ether extract content of pineapple leaves will increase.

Effect of Treatment on Crude Fiber Content of Fermented Pineapple Leaf

The results of the analysis of variance showed that there was a very significant interaction ($P < 0.01$) between *Aspergillus niger* levels and fermentation duration on the crude fiber content of fermented pineapple leaves. The combination of *Aspergillus niger* level 4% and the duration of fermentation of 0 days resulted in the lowest crude fiber content of 17.42%. This is presumably because the pineapple leaves have undergone a steaming process that can loosen the crude fiber bonds. The combination of *Aspergillus niger* level of 2% and the duration of fermentation of 12 days resulted in the highest crude fiber content, which was 26.11%. This is presumably because the longer the fermentation time, the more mycelium is formed so that the crude fiber will increase. In accordance with the opinion of [4] which stated that the increase in crude fiber in the fermentation process was caused by the accumulation of crude fiber along with the proliferation of *Aspergillus niger* followed by an increase in mycelium. [7] stated that the longer the incubation time, the higher the crude fiber content. The growth of the fungal cell population that breaks down the substrate causes changes in the composition of the substrate due to the loss of dry matter during the incubation period. The loss of dry matter will cause an increase in the crude fiber content of the substrate. This is in line with the research results of [17] that the fermentation of cassava peel for 6 days can increase the crude fiber content from 25.68% to 28.60% along with the rapid growth of fungi.

CONCLUSION

The addition of *Aspergillus niger* levels of 0%, 2%, and 4% with Fermentation time of 0 days, 6 days, and 12 days did not result in interactions on dry matter, organic matter, and crude protein content, but interactions occurred on ether extract and crude fiber content of fermented pineapple leaves. The use of *Aspergillus niger* with a level of 0%

and a fermentation duration of 0 days resulted in a fermented product with the lowest ether extract content, namely 1.09% with a dry matter content of 13.40% and an organic matter content of 92.16%. The treatment combination of *Aspergillus niger* 4% and fermentation duration of 0 days was the best combination in producing the lowest crude fiber content of the fermented product.

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REFERENCES

1. F.T. Farda, E.B. Laconi, and S. Mulatsih, *J. Indones. Trop. Anim. Agric.* 40, 167, 2015.
2. F. Fathul, L. Liman, P. Purwaningsih, and S. Tantalo, in Ed. Ke 6, Penerbit Universitas Lampung, 2020.
3. A. Ringgita, L. Liman, and E. Erwanto, *J. Ilm. Peternak. Terpadu* 3, 175, 2015.
4. N. Azizah, A. Al-bAARI, and S. Mulyani, *J. Apl. Teknol. Pangan* 1, 72, 2012.
5. N.N. Palinggi, U. Usman, K. Kamaruddin, and A. Laining, *J. Ris. Akuakultur* 9, 417, 2014.
6. AOAC, Official Methods of Analysis of AOAC International 18th Edition AOAC International Suite 500 481 North Frederick Avenue Gaithersburg, Maryland, USA, 2006).
7. Wiwik Srilidiya Wati; Mashudi; Artharini Irsyammawati, 1, 45 (2018).
8. A. Jayanegara, R.P. Harahap, S. Suharti, and N. Nahrowi, *IOP Conf. Ser. Mater. Sci. Eng.* 1098, 042101 (2021).
9. M. Landupari, A.H.B. Foekh, and K.B. Utami, *J. Peternak. Indones. Indonesian J. Anim. Sci.* 22, 249 (2020).
10. J. Bestari, A. Thalib, H. Hamid, and D. Suherman, *J. Ilmu Ternak Dan Vet.* 4, 1, 1998.
11. Y. Yanuartono, A. Nururrozi, S. Indarjulianto, and H. Purnamaningsih, *TERNAK Trop. J. Trop. Anim. Prod.* 20, 16 (2019).
12. A.O. Hendawy, S. Sugimura, K. Sato, M.M. Mansour, A.H. Abd El-Aziz, H. Samir, M.A. Islam, A.B.M. Rubayet Bostami, A.S. Mandour, A. Elfadadny, R.F. Ragab, H.A. Abdelmageed, and A.M. Ali, *Fermentation* 8, (2022).
13. A.P. Kusuma, S. Chuzaemi, and M. Mashudi, *J. Nutr. Ternak Trop.* 2, 1, 2019.
14. A. Abrar and A. Fariani, *J. Peternak. Sriwij.* 7, 40, 2019.
15. M. Ahmad, F.M. Suhartati, and T. Widyastuti, *ANGON J. Anim. Sci. Technol.* 2, 106, 2020.
16. M.A. Al-Arif, L.T. Suwanti, A.S. Estoepangestie, and M. Lamid, *KnE Life Sci.* 3, 338, 2017.
17. S. Sandi, *J. Penelit. Sains* 15, 15218, 2012.