EFFECT OF SIGER RICE FROM WAXY CASSAVA (*Manihot esculenta*) ON OLIGOSACCHARIDE LEVELS AND CHEMICAL BLOOD PROFILES IN MICE

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Abstract. Siger rice is the Lampung term "Beras Singkong Segar" which is an artificial rice made from cassava. Siger rice is made from cassava which contains high amylose. The amylose come out from the granules to bind water during the cooking and easily release the water and then the rice becomes hard and chewy. Therefore, siger rice will be made from waxy cassava having no amylose to produce soft siger rice after being cold. The aim of this research was to investigate the effects of siger rice from waxy cassava on oligosaccharide levels and blood chemical profile in mice. The research was conducted in complete randomized block design with four replications. Siger rice was made from waxy cassava using a single screw extruder. The results showed that siger rice has characteristics of white color, slightly cassava flavored, containing water (9.81%), ash (0.47%), fat (0.90%), protein (2.13%), crude fiber (4.79%), carbohydrates (81.90%), and glycemic index 30. The processing of waxy cassava into siger rice can reduce levels of raffinose, stachyose, verbascose, and oligosaccharides 81.44, 92.09, 79.27, and 83.99%, respectively. Giving siger rice to mice for 28 days had no negative effects on erythrocyte, hemoglobin, leukocyte, and hematocrit of mice.

Key words: Haematological, *Manihot esculenta*, oligosaccharide, siger rice, waxy cassava

I. INTRODUCTION

Increased economic status of the community resulted in increased food consumption exceeds the body's needs. This condition causes nutritional problems that cause various degenerative diseases, especially diabetes (Waqas et al., 2017). Therefore, alternative food ingredients need to be made has a good taste, low glycemic levels, low calorie content, and rich in content food fiber and bioactive components. One alternative is siger rice.

Siger rice is the Lampung people's term "Beras Singkong Segar " which is artificial rice made from cassava. Siger rice products were developed in Lampung for support food diversification programs in reducing dependence on rice rice (Henita et al., 2018). Siger rice is made in the form of grain, color, and taste like rice so that it can accepted by the community and does not conflict with the Indonesian eating tradition. Siger rice as Lampung's superior local food since 2015 has been instructed to become a food menu served in offices and hotels in Lampung Province based on the Governor's Instruction Lampung No: 521/1159/11.06/2015.

Siger rice is made from cassava flour which has a very high amylose content. In the process of making siger rice, amylose gelatinized due to the heating process so the dough becomes sticky and hard to be molded into rice grains. Waxy cassava plants can be seen in Figure 1 High amylose content in siger rice products cause amylose to come out of the starch granules to bind a certain amount of water during the cooking process and will easily release the water again at the room temperature until

the rice becomes hard. Therefore in this research will be made siger rice from waxy cassava clone (*Manihot esculenta*) that do not contain amylose to produce soft siger rice after being cold.The aim of this research was to know the process of making siger rice from waxy cassava and the effects of siger rice on oligosaccharides levels and blood chemical profile in mice.

(a) **(b)** **(c)**

Figure 1. Waxy cassava (*Manihot esculenta*). (a) petiole and shoots of plant, (b) plant stem, and (c) iodine staining tuber

II. MATERIALS AND METHODS

2.1. Place and time of research

This research was carried out in Laboratory of Quality Testing of Agricultural Product, Department of Agricultural Product Technology, Faculty of Agriculture, Lampung University, and Laboratoirum of Pathology of Regional Veterinary Investigation and Testing of Lampung Province in May to October 2019.

2.2. Materials

The waxycassava samples were collected from the Integrated Field Laboratory of the Faculty of Agriculture, Lampung University, Bandar Lampung. The oligosaccharide standards, raffinose, stachyose and verbascose, sodium acetate, acetic acid, HCl, TLC plate, n-propanol, ethyl acetate and thiobarbituric acid were purchased from Sigma Chemical Company, St. Louis MO, USA.

The laboratory apparatus used in this research are extruder granular machine, blender, mixer, scales, sieve, pan, basin, strainer, screw machine, stove, pot, EDTA tube, hematology analyzer, cage pen, drink bottle and feed container mice.

2.3. Research methods

The research was conducted in complete randomized block design with 4 replications. The study was conducted using 24 male mice divided into 6 groups, namely K1 (78.27% corn starch), K2 (78.27% siger rice), P1 (30, 97% siger rice), P2 (40.97% siger), P3 (50.97% siger rice), P4 (60, 97% siger rice). Each group consists of 4 mice. Mice were treated with a ration composition consisting of corn starch, siger rice, mineral mix, vitamin mix, oil and casein. Furthermore, the mice were kept for 28 days and fed and drank in *ad libitum*. The similarity of data were tested by Bartlet and the addition of data was tested by Tuckey. Data were analyzed by means of variation to obtain the error estimator andsignificance test to determine whether there was any difference between treatments. Further the data were analyzed by the least significant difference at 5% level.

2.4. Implementation of Research

2.4.1. Cassava Flour

The raw materials used in making siger rice are cassava flour according to method of Subeki et al., 2016. The cassava roots were peeled manually with a knife, washed with tap water and shredded using a grater machine into slurry. Cassava is then soaked for 1hour and then washed and squeezed until obtained cassava filtrate and pulp. The filtrate is allowed to stand untiltapioca precipitate was obtained and dried in an oven at 60^{o}C and ground into flourtapioca. The cassava pulp is dried in an oven at 60^{o}C until it is dried and ground intocassava pulp flour.

2.4.2. Siger Rice

Making siger rice are cassava flour and tapioca flour according to method of Subeki et al., 2016. Making siger rice is done by mixing cassava flour and tapioca (4: 1) with the addition of 30% water. The ingredients are mixed with a mixer and then steamed at 90^{o}C for 30 minutes. Then the material is put in a single screw of extruder machine at 45 rpm rotation, 40 rpm cutting blade rotation, with a rice molding roll ellips shaped 6 mm long and 2 mm thick to obtain siger rice grains. Next dried in an oven at 60^{o}C until dry with a water content of less than 13%. The process of making siger rice from waxy cassava can be seen in Figure 2.

Figure 2. The process of making siger rice from waxy cassava. (a) cassava tuber, (b) grinding, (c) drying, (d) sieving, (e) mixing, (f) steaming, (g) extruding, and (h) siger rice

2.4.3. Oligosaccharides

Oligosaccharide extraction was carried out based on the Somiari and Balogh (1993). A total of 5 g of sample was added 50 mL of 70% ethanol then placed in an incubator shaker at 130 rpm for 13 hours. The extract is filtered and the residue obtained is washed with 25 mL 70% ethanol. The filtrate is then dried by vacuum evaporator at 40^{o}C until dry and then dissolved in 5 mL of distilled water. Separation of oligosaccharides was carried out with preparative TLC of cellulose-G. A total of 5 mL of each sample was placed on TLC and developed with n-propanol, ethyl acetate, and distilled water

(6:1:3) (Tanaka et al., 1975). The plates are then sprayed with 1% a-naphthol reagent and dried in the oven. Spots were compared to standard verbascose, stachyose and raffinose spots. Each spot of separated oligosaccharide on TLC was scraped off to take cellulose-G powder and then dissolved in 2 mL of distilled water and stored overnight.The extract was then filtered and each oligosaccharide obtained was analyzed by the Tanaka et al. (1975). A total of 1 mL of each oligosaccharide added 1 mL of 0.2 M thiobarbituric acid and 1 mL of HCl. The filtrate is then boiled in a water bath for 6 minutes. After cooling, filtrate was measured with a spectrophotometer at 432 nm.

2.4.4. Effects of Siger Rice on Blood Profiles of Mice

Mice used were male mice aged 2 months and free of infectious diseases. Mice adapted for 7 days in animal cage, Department of Agricultural Products Technology, Faculty of Agriculture, University of Lampung. Mice will be divided into 6 groups with each group consisting of 4 tails placed in separate cages. The treatment given to the mice is in the form of a siger rice ration composition, with no corn starch and no siger rice. The composition of treatment as in Table 2. Mice will be nourished and given treatment for 28 days and given feeding and drinking ad libitum. Observations were made on the 28th day of the blood profile and histology of the liver and kidneys of the mice. Prior to the preparation of treatment rations should be done proximate analysis of siger rice. Proximate analyzes were carried out on water content, ash, fat, protein, crude fiber and carbohydrates. The results of proximate ricesiger analysis can be seen in Table 1. Feed composition in mice can be seen in Table 2.

Table 1. Results of proximate analysis of siger rice.

Parameter	Percentage (%)
Water	9,81
Ash	0,47
Fat	0,90
Protein	2,13
Crude fiber	4,79
Carbohydrate	81,90

Composition of ration given with the calculation adjusted AOAC method (1990) with the following formula:

$$\text{Protein} = X = \frac{1{,}60 \times 100}{\%\ \text{N sample}}$$

$$\text{Water} = \frac{5 - (X \times \%\ \text{water content})}{100}$$

$$\text{Mineral mix} = \frac{5 - (X \times \text{ash content})}{100}$$

$$\text{Soybean Oil} = \frac{8 - (X \times \text{fat content})}{100}$$

$$\text{Selulosa} = \frac{1 - (X \times \text{crude fiber})}{100}$$

Vitamin = 1

Corn starch = 100 – (Protein + oil + vitamin + mineral + water)

Table 2. Feed composition as treatment in mice.

Composition (g/100 g)	Treatment (g)					
	K1	K2	P1	P2	P3	P4
Corn starch	78,27	-	47,3	37,3	27,3	17,3
Rice siger	-	78,27	30,97	40,97	50,97	60,97
Casein	8,42	8,42	8,42	8,42	8,42	8,42
Soybean oil	7,55	7,55	7,55	7,55	7,55	7,55
Mineral mix	4,76	4,76	4,76	4,76	4,76	4,76
Vitamin mix	1	1	1	1	1	1
Total	100	100	100	100	100	100

Observations were made on blood chemistry profiles of erythrocytes, leukocytes, hematocrit, and hemoglobin.

III. RESULT AND DISCUSSION

3.1. Oligosaccharide

The levels of oligosaccharides, raffinose, stachyose, and verbascose on waxy cassava and processed products can be seen in Table 3. Decreasing levels of raffinose, stachyose, verbascose, and oligosaccharides from cassava which is processed into cassava flour are 21.95-47.30% and siger rice 79.27-92.09%. Verbascose is the highest level of oligosaccharide compounds in cassava flour and siger rice. Previous research also shows that verbascose is the dominant compound in *Vignamungo*, *Cajanuscajan*, and *Phaseolusmungo* (Navikul and D'Appolonia, 1978; Reddy et al., 1984; Jood et al., 1985; Mulimani and Devendra, 2000).

Table 3. Effect of cassava processing on the levels of oligosaccharides (g/100 g).

Treatments	Raffinose		Stachyose		Verbascose		Oligosaccharides	
		% decrease		% decrease		% decrease		% decrease
Cassava	1.67±0.09		2.53±0.05		4.10±0.07		8.31±0.08	
Flour	0.88±0.19	-47.30	1.75±0.13	-30.83	3.20±0.24	-21.95	5.82±0.12	-29.96
Siger rice	0.31±0.01	-81.44	0.20±0.05	-92.09	0.85±0.16	-79.27	1.33±0.09	-83.99

Processing of waxy cassava into flour can reduce levels of raffinose, stachyose, verbascose, and oligosaccharides 47.30, 30.83, 21.95, and 29.96%, respectively. Previous studies have shown that reductions in raffinose levels also occur in the processing of flour of *Cajanuscajan*, *Cicerarietinum,Phaseolus vulgaris, Viciafaba,* and *Canavaliaensiformis* (Jood et al., 1985; Oboh et al., 2000; Siddhuraju and Becker, 2001). The flour processing of *Cajanuscajan, Vignamungo,* and *Phaseolus vulgaris* also shows a decrease in stachyose levels (Jood et al., 1985).

Decreased oligosaccharide levels into cassava flour due to the process of soaking and washing with water. Making cassava flour is done by soaked and washed with water and then dried. Previous Research shows that oligosaccharide compounds can be removed by soaking and washing with water. The loss of oligosaccharide compounds can be increased by a longer soaking process (Upadhyay and Garcia, 1988).

The processing of waxy cassava into siger rice can reduce levels of raffinose, stachyose, verbascose, and oligosaccharides 81.44, 92.09, 79.27 and 83.99%, respectively. The stachyose content in siger rice is reduced to 92.09%. Previous studies have shown that cooking of soybeans and *Mucuna pruriens* var. utilis can reduce stachyose levels up to 95-98% (Mulimani et al., 1997; Janardhanan et al., 2003).

The processing of siger rice from waxy cassava can reduce oligosaccharide levels. This is because the heating process in making siger rice can cut the oligosaccharide compounds into di- and monosaccharides. Thus, the results of this study indicate that the heating process during the making siger rice is more effective than soaking and washing in making cassava flour in decreasing levels of raffinose, stachyose, verbascose, and oligosaccharides.

3.2. Number of Erythrocytes

Based on the observation on blood profile of mice, it is known that the amount of erythrocytes of mice after giving feed can be seen in Figure 3.

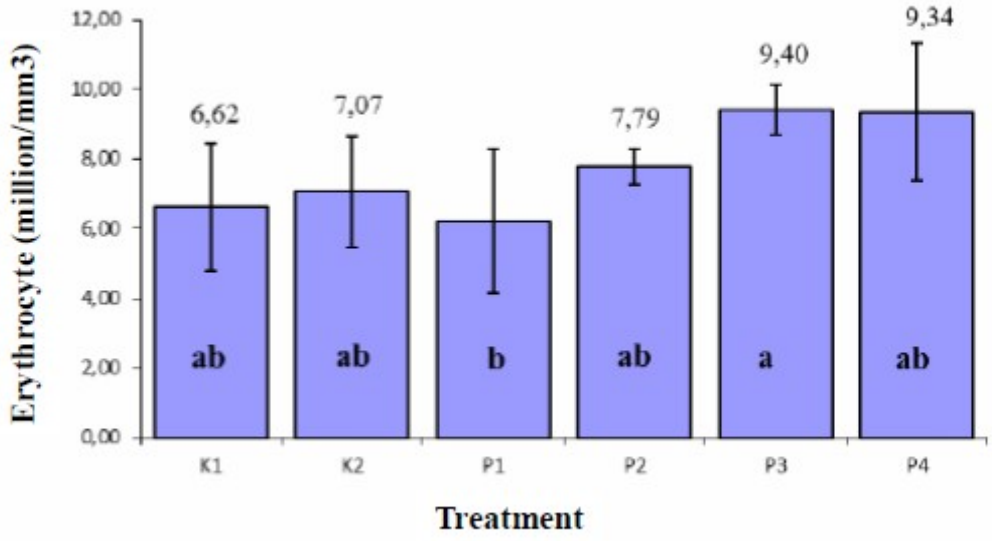

Figure 3. Number of erythrocytes of mice at various treatments. The same letter shows no significant difference at the level of 5%. K1 (corn starch 78.27%), K2 (siger rice 78.27%), P1 (siger rice 30.97%), P2 (siger rice 40.97%), P3 (siger rice 50.97%), and P4 (siger rice 60.97%) in feed composition.

The result of variance analysis showed that the treatment of siger rice ration fromc assava did not significantly affect the amountof erythrocytes of mice. Treatments that had an erythrocyte count below the normal erythrocyte count were treatment of K1 and P1, respectively 6.62 and 6.22 million/mm3. While other treatments were still in the range of normal erythrocyte counts, ie treatment of K2, P2, P3, P4 were 7.07, 7.79, 9.40 and 9.34 million/mm3, respectively. The number of normal erythrocyte mice is 6.86 - 11.7 million/mm3 (Kusumawati, 2004).

For treatments having abnormally lower amounts of erythrocytes, the treatment of K1 and P1 is thought to be due to other factors such as the physiological condition of the mice. Each mice has a normal range of different erythrocytes depending on the strain, physiological condition or sex (Subekti et al., 2013). Several factors may affect the physiological conditions in animals such as environmental temperature, nutritional qualityof the feed, body fluid balance and breeding (Ciaramella et al., 2005).

Cyanide will enter the blood stream through several routes. Generally cyanide compounds come in along with food. In the digestive tract, the cyanide ion is easily absorbed and then distributed into the blood, liver, kidneys, brain, and so forth (Buck et al., 1976; Sylvester et al., 1983). Cyanide in the blood can be found, among others, inerythrocytes and hemoglobin (Cohen and Guzzardi, 1984; Mc Millan and vodoba, 1982). However it can be seen that most of the treatments in this study did not affect the amount of erythrocytes of mice. In this case it can occur because the mice used in the study are healthy mice and are not conditioned in anemia or anything, so that the mice have a limit in

producing red blood cells according to their needs (Metha and Hoffbrand, 2006). Also suspected also because the content of hydrogensainida in siger rice used for 0.02 mg/g and not so affect. So the results of this study showed that the treatment did not affect the amount of erythrocytes of mice seen from the amount of erythrocytes that are still within the normal range.

3.3. Number of Leukocytes

Based on the observation on blood profile of mouse, the number of leukocytes of mice after giving feed can be seen in Figure 4. Leukocytes are the blood units that are active in the body's defense system against the attack of pathogens, toxic substances, and getrid of damaged and abnormal cells (Kelly,1984; Guyton and Hall, 1997). Leukocytes have a primary function of protecting the body from invading foreign organisms (Besa, 1992).White blood cell response can be in the form ofa decrease in the total number of leukocytes or called leukopenia as well as an increase in the total number of leukocytes or called leukocytosis (Mayer and John, 1998).

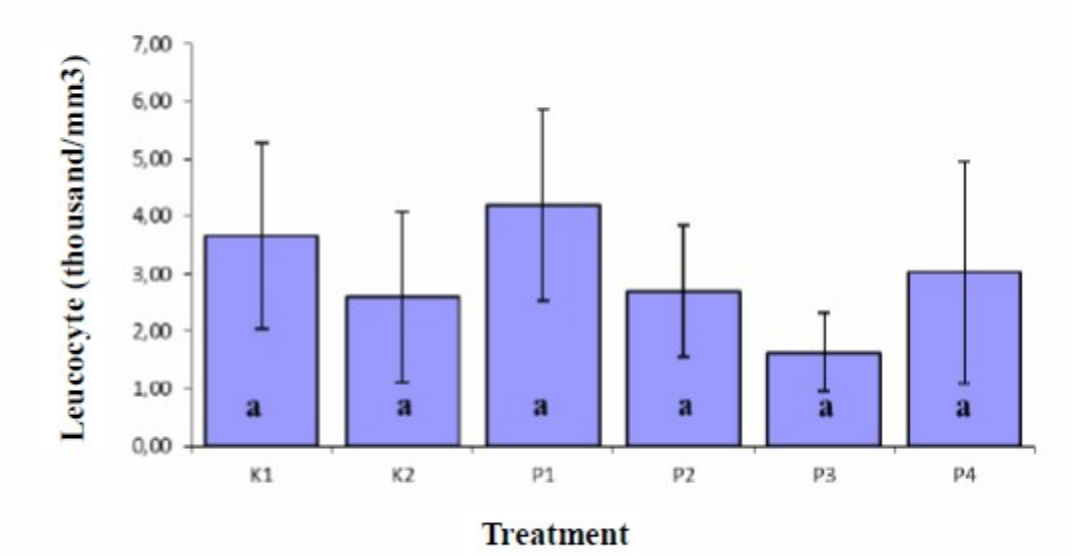

Figure 4. Number of leukocytes of mice at various treatments. The same letter shows no significant difference at the level of 5%. K1 (corn starch 78.27%), K2 (siger rice 78.27%), P1 (siger rice 30.97%), P2 (siger rice 40.97%), P3 (siger rice 50.97%), and P4 (siger rice 60.97%) in feed composition.

The result of variance analysis showed that the treatment of siger rice from cassava did not significantly affect the number of leukocytes of mice. However, when viewed from the number of leukocytes of mice, all treatments of rationing included controls had leukocyte counts below the normal leukocytesof mice ie K1, K2, P1, P2, P3, and P4 were 3.67, 2.60, 4.20, 2.70, 1.63, and 3.03 thousand/mm3, respectively. The number of leukocytesof normal mice is 6 - 15 thousand/mm3(Fahrimal et al., 2014).

If leukocytes as a defense of the body decreases it will facilitate foreign microorganisms to infect the body of mice (Stephen and Wiliam, 2010; Yuliarti, 2009). Leukocytes are active units in the body's defense system so that the number will increase if there is infection (Kimball, 1988). The low number of leukocytes of mice in each treatment is thought to be due to a lack of response from the body's defense system of mice to the presence of a hydrogen cyanide toxic substance in the treatment of rationing. Research conducted by Mahawati et al (2006) showed that the tendency of high leukocyte counts or even close to the maximum value indicates that the body's response to toxic substances. However, according to Aboderin and Oyeyato (2006) data on the total leukocyte count alone can not provide specific information regarding the status of the immune system of animals, hence the need for further calculation of the number of each type of leuocyte cell.

The low leukocytes of mice can also be affected by physiological changes in the body of mice. Physiological changes that occur in the animal body is a factor that can affect the picture of blood. These physiological changes include internal and external physiological changes. Internal physiological changes include age, nutritional status, exercise, health, stress, blood production process, estrus cycle, body temperature. While external physiological changes include germ infection, fractures, changes in environmental temperature, sanitation and so on (Banks, 1986).

3.4. Hemoglobin levels

Based on the observation on the blood profile of mice, hemoglobin content of mice after giving feed can be seen in Figure 5.

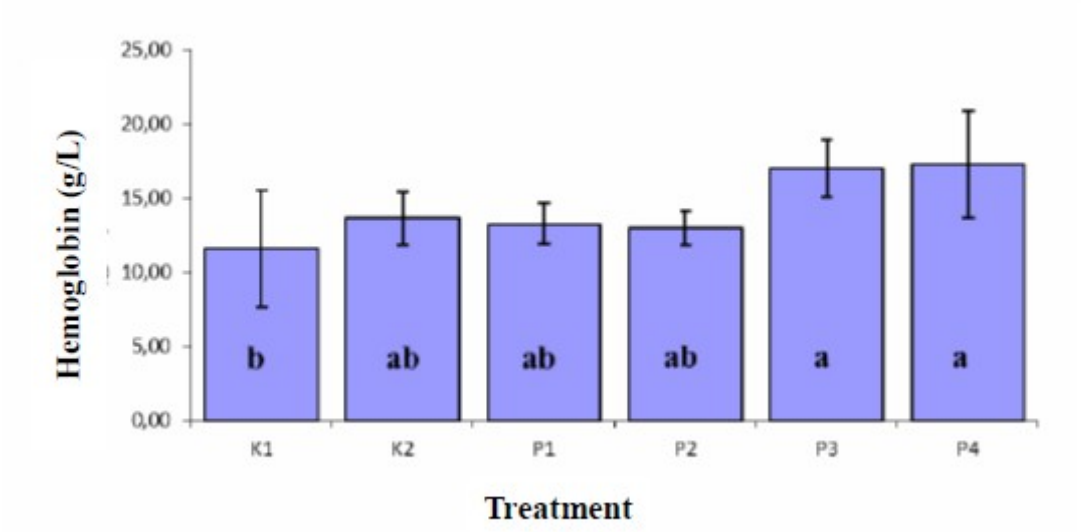

Figure 5. Number of hemoglobin of mice at various treatments. The same letter shows no significant difference at the level of 5%. K1 (corn starch 78.27%), K2 (siger rice 78.27%), P1 (siger rice 30.97%), P2 (siger rice 40.97%), P3 (siger rice 50.97%), and P4 (siger rice 60.97%) in feed composition.

The result of variance analysis indicated that the giving of siger rice from waxy cassava had no significant effect on mice hemoglobin level. Levels of hemoglobin mice treated with siger rice were in the range of normal hemoglobin levels in the treatment of K1, K2, P1, P2 and P3 were 11.60g/L, 13.63 g/L, 13.27 g/L, and 12.97 g/L, respectively. While in treatment P3 and P4 have the average of high hemoglobin level in sequence equal to 17,00 g/L and 17,27 g/L and is outside the level of normal mouse hemoglobin. The range of mice hemoglobin level is 10-14 g/mL (Ganong,1985).

Hemoglobin has four polypetide chains and four heme prosthetic groups in which the group has iron atoms in the form of ferro (Fe^{2+}) (Marieb, 2005). When a cyanide ion is present in the body, it binds a heme prosthetic group in which the heme prosthetic is a cofactorin cytochrome oxidase (Jensen et al., 1984). Cyanide is generally rapidly distributed throughout the body after absorption and is rapidly absorbed rapidly into the blood stream.Then there will be oxygenation of high oxygen levels in the blood because cyanide reacts with ferric iron from cytochrome oxidase and forms a high cyanide cyanide. Mean while, hemoglobin will lose the ability to free oxygen (electron transport system) (Osweiler et al.,1976).

Oxygen is one of the major substances needed in chemical reactions in cells. Every cell needs oxygen to convert food energy to ATP (Adenosine Triphospate) to be used in the work of each cell (Guyton and Hall, 2012; Saleh, 2014). According to Elizabeth (2009) when the network is not able to absorb oxygen then the condition is called historiik hypoxia which one of them caused by cyanide poisoning. Cyanide in the body will activate some oxidative enzymes especially cytochrome oxidase by binding to ferric heme group. It is also expressed according to Solomonson (1981) acknowledged

that cyanide toxicity is primarily due to decreased oxygen utilization in the tissues, resulting in a histotoxic state of anoxia.

Because hemoglobin is not able to free oxygen, so the network is difficult to absorb the oxygen required by metabolism, which is one of them because of the cyanide effect. The impact of network sustainability that is not able to absorb oxygen is the existence of tissue damage that starts from cell degeneration.

3.5. Hematocrit Value

Based on the observation on the blood profile of mice, hemoglobin content of mice after giving feed can be seen in Figure 6.

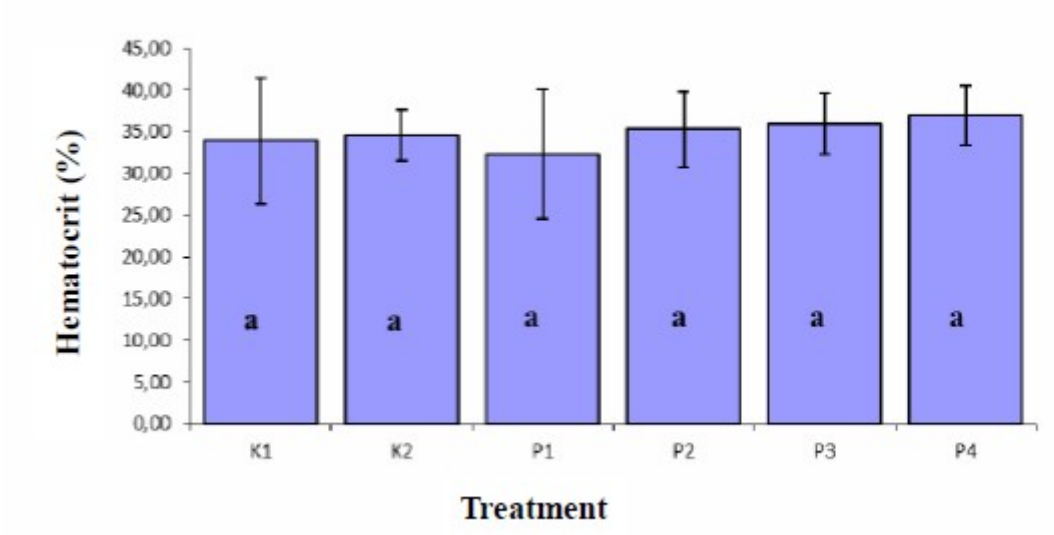

Figure 6. Hematocrit value of mice at various treatments. The same letter shows no significantdifference at the level of 5%. K1 (corn starch 78.27%), K2 (siger rice 78.27%), P1 (sigerrice 30.97%), P2 (siger rice 40.97%), P3 (siger rice 50.97%), and P4 (siger rice 60.97%) infeed composition.

The results of variance analysis showed that the treatment of siger rice ration from waxy cassava did not affect the hematocrit value of mice. However, when viewed from the hematocrit value of mice all treatments have hematocrit values below the normal hematocrit of mice. The treatments of K1, K2, P1, P2, P3 and P4 had hematocrit values of 34,00,34,67, 32,33, 35,3, 36,00 and 37,00%, respectively. While mice have an average hematocrit value of 40-45% (Guyton, 1985).

Hematocrit is the percentage of red blood cells in 100 mL of blood. The hematocrit value is also an indicator of anemia in animals (Guyton, 1995). However, anemia is not always indicated by low hematocrit values. This is as explained by Subekti et al (2013) that low hematocrit values are not always accompanied by anemia. The low value of hematocrit can be caused by increased blood plasma volume even though the amount of erythrocytes is in the normal category. This study showed that the average number of erythrocytes of mice is still within the range of normal erythrocytes.

So far, siger rice is made from ordinary cassava flour which has a high amylose content. In the process of making rice siger, amylose is gelatinized due to heating so that the dough becomes sticky and cannot be molded into rice grains (Subeki et al., 2016). Therefore, analog rice producers add other ingredients such as corn flour to reduce the stickiness of the dough so that it can be made into rice grains. In this study siger rice was made from waxy cassava contained high amylopectin and did not amylose. Therefore, when making siger rice, waxy starch does not occur excessive gelatinization and the dough is not sticky and is more easily formed into rice grains. Siger rice made from waxy cassava has a soft texture and is not hard after being cold. Giving of siger rice from waxy cassava containing

high amylopectin and no amylose to mice for 28 days had no negative effects on erythrocyte, hemoglobin, leukocyte, and hematocrit of mice.

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