Evaluation of Siger Rice from Waxy Cassava (*Manihot Esculenta Crantz*) in Rat

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Abstract: This study aimed to evaluate the toxicological potential of siger rice from waxy cassava in the rat. Siger rice is a Lampung people's term "Beras Singkong Segar" which means artificial rice made from cassava. At first, siger rice was made from native cassava containing high amylose. The study was conducted in a randomized block design with ten replications. Siger rice is made from waxy cassava using a single screw extruder. Siger rice fed to rats for 21 days had feed conversion of 4.26 and coefficient digestibility of 67.16%. Moreover, rat fed siger rice had no negative haematological effect of packed cell volume, red blood cell and white blood cell with each successive value of 24.21%, 4.17x106/mL, and 5.86x106/mL. Rats fed siger rice showed the serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase, albumin, and bilirubin with each successive value of 250.17 IU/L, 42.19 IU/L, 0.21 mg/dL, and 3.05 mg/dL no significant difference with rat fed control. Further pathological investigation revealed that the liver had no necrotic lesion.

1 INTRODUCTION

The increased economic status of the community caused by the increased food consumption exceeds the body's needs. This condition causes nutritional problems causing various degenerative diseases, especially diabetes (Lathifah, 2017). Therefore, alternative food ingredients need to be made to have a good taste, low glycemic levels, low-calorie content, and high levels of dietary fibre and bioactive components. One alternative is siger rice.

Siger rice is the Lampung people's term "Beras Singkong Segar", artificial rice made from cassava. Siger rice was developed in Lampung to support food diversification programs in reducing dependence on rice (Henita, et al., 2018). Siger rice is made in the form of grain, colour, and tastes like rice to be accepted by the community and does not opposite with the Indonesian eating habits. Siger rice, as Lampung's fine 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.

At first, siger rice is made from native cassava flour containing high amylose content. In the processing of siger rice, amylose gelatinized due to the heating process. Therefore, the dough becomes sticky and hard to be extruded into rice grains. High amylose content in siger rice cause amylose to leak out from the starch granules to bind an amount of water during the cooking process and it will easily rerelease the water until the rice becomes hard. Therefore, this research will made siger rice from a waxy cassava clone (*Manihot esculenta* Crantz) containing high amylopectin and free amylose to produce soft siger rice after being cold. The objective of this research was to evaluate the toxicological potentials of the siger rice from waxy cassava in rats.

This research was carried out in the Laboratory of Quality Testing of Agricultural Product, Faculty of Agriculture, Lampung University, and Laboratoirum of Pathology of Regional Veterinary Investigation and Testing of Lampung Province from May to October 2020.

The waxy cassava samples were collected from the Integrated Field Laboratory of the Faculty of Agriculture, Lampung University, Bandar Lampung. The Wistar rat of 2 months old male were obtained from the Regional III Center for Veterinary Investigation and Testing of Lampung Province. Corn oil, CMC, corn starch, vitamin mixtures, mineral

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mixtures, casein, methionine, choline, formalin, xylol solution, alcohol, paraffin, aqua dest, Harris's Hematoxylin and Eosin. The tools used in this research are extruder, grinding, hydraulic press, hammer mill, blender, mixer, analytical balance, sieve, filter, trash, heater, EDTA tube, Hematology Analyzer, rat cage, and drinking bottle.

2 RESEARCH METHODS

The research was conducted in a complete randomized block design with ten replications. The study was conducted using 20 male Wistar rats divided into control and siger rice diets. Each group consists of 10 rats. Rats were treated with a composition of casein, CMC, corn oil, corn starch, siger rice, mineral mix, vitamin mix, DL-methionine, choline, and water. Furthermore, keep the rats for 21 days and be fed *ad libitum*. Bartlet tested the similarity of data, and Tuckey tested the addition of data. Data were analyzed utilizing variation to obtain the error estimator and significance test to determine whether there was any difference between treatments. The data analyzed by students t-test, significance was accepted at $P<0.05$ (Zar, 1984).

2.1 Implementation of Research

2.1.1 Cassava Flour

Cassava flour is made from waxy cassava (Subeki, et al., 2016). The cassava tubers were peeled manually with a knife, washed with tap water and crushed. The crushed cassava was then pressed using a hydraulic press. The pressed pulp was later subjected to the oven at 60ºC and ground into cassava flour.

2.1.2 Siger Rice

Siger rice made from cassava four according to the method of Subeki (Subeki et al., 2016). The making of siger rice is done by mixing cassava flour with the addition of 30% water. The dough ingredients are mixed with a mixer and then steamed for 30 minutes. The material is then put in a single screw of extruder machine at 45 rpm rotation, 40 rpm cutting blade rotation, with dyes a siger rice hole of ellipse with 6 mm long and 2 mm thick to obtain siger rice grains. Siger rice was then dried in an oven at 60ºC until the moisture of less than 13%.

2.1.3 Sample Analysis

Analysis of protein content was determined by the micro-Kjeldahl method (AOAC, 1990). Siger rice 0.5 g is hydrolyzed with 10 mL H_2SO_4 using $CuSO_4.5H_2O$ catalyst until the solution becomes grey-white. The hydrolyzed filtrate 10 mL were distilled with 15 mL 40% sodium hydroxide for 10 minutes into 20 mL 2% boric acid containing three drops of indicator solution (a mixture of 0.018 g bromocresol green and 0.016 g methyl red in 100 mL water). The distillate was then titrated with 0.01 M HCl. The nitrogen (N) content is calculated from the titer and crude protein is obtained by multiplying the nitrogen content by the conversion factor Nx6.25. The blank determination is carried out simultaneously. Analysis of moisture, ash, crude fiber, fat, and carbohydrates using the AOAC method (AOAC, 1990).

2.1.4 Nutritional Quality

The analysis of the nutritional quality of siger rice in Wistar rats divided into two groups. Rat in each group kept in individual wire cages at room temperature. The feed was formulated by replacing cornflour with siger rice (Autor, 1993). Feed intake, daily weight gain, feed efficiency and digestibility were measured during the experiment. At the end of the 21-day trial period, all rats were killed by asphyxiation and blood was taken by heart puncture, and the serum was then stored at -20ºC until it being analyzed. The liver and kidneys remove for further examination. The feed composition of basal siger rice flour in the rat can be seen in Table 1.

Table 1. Feed composition of basal siger rice flour (g/1000 g)

Sampel	Diet	
	Standard (AIN-76)	Siger rice
Casein	200	146.4
CMC	50	43.35
Corn oil	180	176.65
Corn starch	539	130.25
Siger rice	-	500
Mineral mix	135	133.15
Vitamin mix	10	10
dl Methionine	3	3
Colin	1	1
Water	25.8	0
Total	1043.8	1043.8

2.1.5 Haematology Tests

Haematological tests on the number of packed cell volume (PCV), red blood cells (RBC) and white blood cells (WBC) were conducted using the conventional method (Aning, 1998). Biochemical tests on albumin, bilirubin, Glutamate Oxaloacetate Transaminase (GOT), and Glutamate Pyruvate Transaminase (GPT) were carried out by conventional methods (Mokadi, et al., 1989).

2.1.6 Histopathological Tests

The histopathological test was carried out by taking the liver and kidneys of each rat and then stored in a 4% formalin-saline solution. After dehydration and embedding in paraffin wax, 5-mm sections were cut and stained for examination in the microscope. The internal organs were inspected for morphological lesions.

3 RESULTS AND DISCUSSION

The results showed that the bodyweight of the rats increased every day in the group of mice were fed control and siger rice diets. The increase in body weight indicates good feed conversion efficiency and overall performance. It is a good indicator that the diet fed to mice has a high nutritional quality (Van, 1999). Table 2 shows that the control diet-fed mice showed higher body weight gain than the siger rice diet-fed mice (Figure 1). The higher growth rate in mice fed the control diet was due to the higher feed intake (Aning, et al., 1998). The mice fed the control diet had a higher feed intake than those fed the siger rice diet. It shows the amount of feed consumption plays a vital role in the growth of the rat (Aning, et al., 1998).

Table 2. Nutrient utilization and digestibility of rats fed siger rice flour

Sample	Control	Siger Rice
Daily weight gain (g)	2.72 ± 0.17^a	2.05 ± 0.33^b
Daily feed intake (g)	9.53 ± 0.12^a	8.74 ± 0.83^a
Feed:Gain ratio	3.50 ± 0.71^a	4.26 ± 0.40^a
Daily excretion (g)	3.12 ± 0.22^a	2.87 ± 0.31^a
Digested amount (g)	6.41 ± 0.73^a	$5.87 + 0.88^a$
Coeficient digestibility (%)	67.26 ± 5.25^a	67.16 ± 6.63^a
Dry matter digestibility (%)	$68.56 + 5.38^a$	$66.32 + 6.95^a$

Values are mean + S.E (n =3). Means with the same superscript letter(s) along the same row are not significantly different (P>0.05).

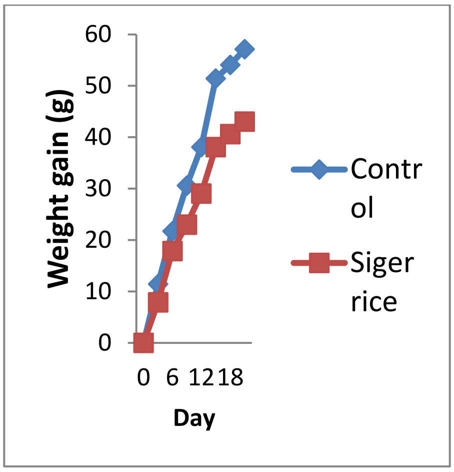


Figure 1. Growth curve of rats fed siger rice

The rats fed the control diet had a lower ratio, than those fed by the siger rice diet. It shows that the low growth in rats fed the siger rice is not due to poor nutrient quality but the low amount of feed intake (Aning, et al., 1998). However, rats efficiently converted the little feed consumed into weight gain (Aning, et al., 1998).

Digestibility results of siger rice are shown in Table 2. There was no significant difference in the digestibility coefficient of control and siger rice. The value of this digestibility coefficient is higher than cassava processed by fermentation but lower than cooked casein by 91.2% (Bressani, 1999). It shows that the protein in the feed is very easy to digest. This high digestibility indicates a reduction in tannins during cassava processing.

The results showed no significant difference between dry matter digestibility in the control and siger rice diet. The dry matter digestibility of siger rice is lower than that of raw sweet potatoes (85.4%) and cooked sweet potatoes (90.4%) (Canopy, Tamiya, 1977). These results indicate that the siger rice-based diet has high digestibility. Therefore, processed siger rice by extruders can improve the nutritional quality of the product.

Table 3 shows the levels of GPT, GOT, bilirubin, and albumin in the serum of rats fed control and siger rice. The results showed no significant difference between GOT levels in the control (247.11 IU/l) and siger rice (250.17 IU/l) diets. There was no increase in GOT in rat fed siger rice due to no secretion of toxins during the processing of siger rice. Aflatoxin is a toxic compound that can cause liver damage (Oboh, et al., 2000). Increased blood GOT valuesare attributed to liver damage and possible heart damage [(American Liver Foundation, 1995), (David, 1999)]. There was no significant difference between the GPT levels in the blood of rats fed control (39.14 IU/l) and siger rice (42.19 IU/l) diets. There was no increase in the GPT value in rat fed siger rice, indicating that cassava processing did not produce toxins (Oboh, et al., 2000).

GPT and GOT are enzymes located in liver cells that can leak into the blood circulation when liver cells are injured. GPT levels are a specific indicator of liver inflammation. GOT levels can be increased in a human who has heart disease [(American Liver Foundation, 1995), (American Liver Foundation, 1997), (David, 1999)]. Acute liver disease such as hepatitis shows very high GPT and GOT levels of more than 1000 IU/l (American Liver Foundation, 1997). GPT and GOT levels are highly dependent on the range of liver disease [(American Liver Foundation, 1995), (American Liver Foundation, 1997), (David, 1999)].

Table 3. Serum chemistry and haematological evaluation of rats fed siger rice flour

Sample	Control	Siger rice
GOT (IU/L)	247.11 ± 6.13^a	250.17 ± 5.11^a
GPT (IU/L)	39.14 ± 4.25^a	42.19 ± 2.06^a
Albumin (mg/dL)	0.19 ± 0.14^a	0.21 ± 0.21^a
Bilirubin (mg/dL)	3.26 ± 0.17^a	3.05 ± 0.26^a
PCV (%)	23.68 ± 0.23^a	24.21 ± 2.16^a
RBC (10^6/mL)	3.87 ± 0.18^a	4.17 ± 0.15^a
WBC (10^6/mL)	9.91 ± 0.91^a	5.86 ± 1.03^b

Values are mean ± S.E (n =10). Means with the same superscript letter along the same row are not significantly different (P>0.05).

There was no significant change (P>0.05) in serum bilirubin content of the rats fed siger rice flour diet compared with that of the control diet. This low level of bilirubin in the serum of the rats fed diets containing siger rice flour is an indication that the feed did not cause haemolysis of red blood cells since bilirubin is formed primarily from the breakdown heme [(Lehninger, 1987), (American Liver Foundation, 1997)].

The results showed that the serum bilirubin content of rat fed siger rice of 3.05 mg/dL was not significantly different from the control 3.26 mg/dL. The low bilirubin content in the serum of rat fed siger rice indicates that administration of siger rice does not cause hemolysis of red blood cells. Bilirubin is a compound formed from the breakdown of heme [(Lehninger, 1987), (American Liver Foundation, 1997)].

Rats fed siger rice showed no significant difference in albumin level (0.21 mg / dL) from that of control (0.19 mg/dL). These results indicate that the administration of cassava inhibits albumin biosynthesis in the liver. Albumin is produced primarily in the liver as an indicator of liver function [(American Liver Foundation, 1995), (American Liver Foundation, 1997), (David, 1999)].

The haematology test of rats fed siger rice showed the levels of PCV (24.21%) and RBC (4.17x10^6/mL) were not significantly different from the levels of PCV (23.68%) and RBC (3.87x10^6/mL) control. However, the rat fed siger rice showed significantly lower WBC levels (5.86x10^6/mL) than the control WBC 9.91x10^6/mL. The low white blood cell count in siger rice is due to the bioactive components in cassava, which can inhibit the growth of pathogenic microorganisms. The PCV, RBC, and WBC analysis results are in line with reported in rat fed sorghum (Aning, et al., 1998). These results also align with processed cassava products that do not show haematological effects (Autor, 1993). The histopathological test of the kidney and liver of the rats fed siger rice showed that the diet did not cause any damage to the organ. The pathological studies had shown clearly that the siger rice diet could cause damage to the liver, as earlier indicated by its low serum GPT and GOT activities [(American Liver Foundation, 1995), (American Liver Foundation, 1997), (David, 1999), (Younossi, Mehta, 1998)]. Further pathological investigation showed that the liver had no necrotic lesion. Cell appearance of kidney and liver can be seen at Figure 2 and 3.

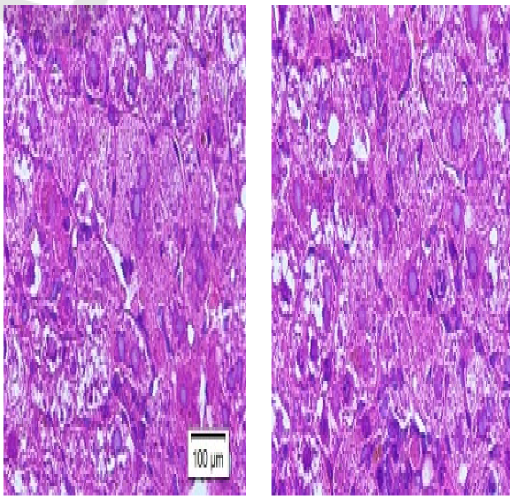
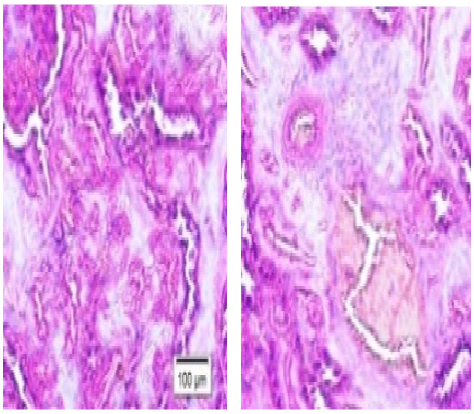


Figure 2. Histological liver of HE stained rat with magnification 100x. (a) control and (b) siger rice



(a) (b)

Figure 3. Histological kidney of HE stained rat with magnification 100x. (a) control and (b) siger rice

4 CONCLUSIONS

Siger rice fed to rat for 21 days had feed conversion of 4.26 and coefficient digestibility of 67.16%. Moreover, rat fed siger rice had no negative haematological effect of packed cell volume, red blood cell and white blood cell with each successive value of 24.21%, 4.17x10^6/mL, and 5.86x10^6/mL.

Rats fed siger rice showed the serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase, albumin, and bilirubin with each successive value of 250.17 IU/L, 42.19 IU/L, 0.21 mg/dL, and 3.05 mg/dL no significant difference with rat fed control. Further pathological investigation revealed that the liver had no necrotic lesion.

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