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Utilization of fermented sago pulp as a source of carbohydrate in feed for Nile tilapia *Oreochromis niloticus*

Pemanfaatan ampas sagu fermentasi sebagai sumber karbohidrat pada pakan ikan nila *Oreochromis niloticus*

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ABSTRACT

This study aimed to evaluate sago pulp fermentation method and its effect on crude fiber content, digestibility, and utilization of sago pulp as a feed raw material for Nile tilapia. Fermentation was done using three different fermenters, i.e. yeast tapai and baker's yeast with five doses of 10 g/kg, 20 g/kg, 50 g/kg,70 g/kg,100 g/kg, respectively, and sheep rumen liquid with five doses of 100 mL/kg, 200 mL/kg, 300 mL/kg, 500 mL/kg and 1000 mL/kg. The incubation time was 0, 24, 72, and 96 hours. In the digestibility test, tilapia (25 g) was stocked at a density of 7 fish/aquarium. Three different diets were applied in quadruplicate, i.e. reference diet (100% reference diet), unfermented sago pulp (AS), and fermented sago pulp (ASF). Three different dietary treatments (in quadruplicate) containing different carbohydrate sources were tested, i.e. cassava flour as a comparion (G), unfermented sago pulp (AS), and fermented sago pulp (ASF). Fermentation of sago pulp with tapai yeast at a dose of 50 g/kg at 72 hours incubation time could reduce crude fiber by 35.76%, neutral detergent fiber (NDF) by 32.68%, and hemicellulose by 60.39%. Fermentation with yeast tapai could significantly increase sago pulp dry matter digestibility by 34% and carbohydrate digestibility by 21%, as well as increase glucose absorption. The growth experiment showed that the use of ASF diets resulted in higher specific growth rate (3.31 ± 0.12%/ day), protein retention (47.34 ± 5.23%) and fat retention (85.58 ± 5.44%) than those of AS dietary. In conclusion, fermentation of sago pulp using yeast tapai at a dose of 50 g/kg at 72 hours incubation could reduce crude fiber content and increase dry matter and carbohydrate digestibilities, so that it can be used as a source of carbohydrates in tilapia diet.

Keywords : carbohydrate, digestibility, fermentation, fiber, Nile tilapia, sago pulp

ABSTRAK

Penelitian ini bertujuan mengevaluasi metode fermentasi ampas sagu dan pengaruhnya terhadap kandungan serat kasar, kecernaan, dan pemanfaatan ampas sagu sebagai bahan baku pakan ikan nila. Fermentasi dilakukan dengan penambahan tiga perlakuan bahan fermentor yaitu ragi tape dan ragi roti ditambahkan dengan dosis masing-masing sebanyak 10 g/kg, 20 g/kg, 50 g/kg, 70 g/kg, 100 g/kg, dan cairan rumen domba yang ditambahkan dengan dosis 100 mL/kg, 200 mL/kg, 300 mL/kg, 500 mL/kg, dan 1000 mL/kg. Lama waktu inkubasi 0, 24, 72, dan 96 jam. Pada uji kecernaan digunakan ikan nila (25 g) yang dipelihara dengan kepadatan tujuh ekor per akuarium. Pada uji ini dilakukan tiga perlakuan pakan dengan empat ulangan, yaitu pakan acuan, ampas sagu tanpa fermentasi (AS), dan ampas sagu fermentasi (ASF). Percobaan dilakukan dengan tiga perlakuan pakan (4 ulangan) dengan tiga sumber karbohidrat yang berbeda yaitu gaplek (G) sebagai pembanding, ampas sagu (AS), dan ampas sagu fermentasi (ASF). Fermentasi ampas sagu dengan menggunakan ragi tape sebanyak 50 g/kg dengan lama inkubasi 72 jam dapat menurunkan serat kasar tertinggi sebanyak 35.76%, dan menurunkan fraksi serat neutral detergent fiber (NDF) dan hemisellulosa masing-masing sebanyak 32.68% dan 60.39%. Perlakuan fermentasi ampas sagu dapat meningkatkan nilai kecernaan bahan sebesar 34%, kecernaan karbohidrat sebesar 21%, serta penyerapan glukosa. Hasil uji pertumbuhan menunjukkan bahwa perlakuan ASF memberikan nilai laju pertumbuhan spesifik (3.31 ± 0.12%/hari), retensi protein (47.34 ± 5.23%) dan retensi lemak (85.58 ± 5.44%) yang lebih tinggi dibandingkan perlakuan AS (P<0.05). Dapat disimpulkan bahwa fermentasi ampas sagu dengan menggunakan ragi tape pada dosis 50 g/kg selama 72 jam dapat menurunkan kadar serat kasar dan meningkatkan kecernaan bahan dan karbohidrat sehingga dapat digunakan sebagai sumber karbohidrat pada pakan ikan nila.

Kata kunci : ampas sagu, fermentasi, ikan nila, karbohidrat, kecernaan, serat

INTRODUCTION

Carbohydrate, in addition to protein and fat, is one of the macronutrients required in feed. The main function of carbohydrate is as an energy source and it is important for the development of a balanced feed for aquatic animals (Kamalam *et al*., 2017). Carbohydrate is also a feed component which shares a fairly large portion, especially for herbivorous and omnivorous fish. For example, the carbohydrate content in feed for Nile tilapia, which is an omnivorous fish, could reach 30–35% (Opiyo, 2014). The common source of carbohydrate used in aquaculture feed is plant-based materials such as wheat pollard, corn, rice, bran, wheat flour, tapioca, *gaplek* (dried cassava root), and sago. In order to fulfill the need for raw materials for feed without being dependent on imported raw materials, it is very important to search for other local raw materials which have the potential to be used as a source of carbohydrate in fish feed, are abundant, and are waste-based or a secondary product so that the price is affordable.

One of the local raw materials which have the potential to be developed as a carbohydrate source is sago pulp. Indonesia has a sago plantation area of approximately 1,128 million Ha or 51.3% of the 2,201 million Ha global sago area with a productivity potential of 30 tons/ha/year which exceeds other food sources such as rice (10–16 tons/ha/year) and corn (8–10 tons/ha/year) by far (Alfons & Rivaie, 2011). In the process of producing sago flour which is the main use of sago palms, waste in the form of sago pulp is produced at a rate of 14 kg wet pulp per kg sago flour (Yusuf, 2018). The main component of sago pulp is starch which varies between 30 and 73% of the dry weight (Adeni, 2013; Lai *et al*., 2013; Muhsafaat 2015). This sago pulp waste is not yet well utilized and could cause environmental issues if it is simply discarded (Tiro *et al.,* 2018; Rosli, 2018; Amos 2010). In addition to its abundance as a secondary product of the sago flour processing industry, sago pulp is also a natural organic material which contains a low ash content. Some efforts to utilize this waste product are for sugar fermentation, as an enzyme, as fertilizer for mushrooms, and livestock feed with the purpose to decrease the pollution caused by the sago industry and also provide an economic solution to the waste management system in sago processing plants (Adeni *et al.,* 2010).

Utilization of sago pulp as a material for fish feed is hindered by its high fiber content which could reach 18% with 16 to 28% of this being lignin which is difficult for fish to digest (Muhsafaat, 2015). The high crude fiber content could affect both the digestibility and palatability of the feed. Therefore, the utilization of sago pulp as an ingredient for fish feed needs to be preceded by decreasing the crude fiber and increasing the nutritional value which could be done through, among others, fermentation technology. Fermentation is a metabolic process where enzymes from microorganisms conduct hydrolysis and other chemical reactions which lead to chemical changes to an organic substrate (Sadh *et al*., 2018). Fermentation to foodstuff results in a number of benefits such as an improved quality both from the nutritional aspect and from the digestibility aspect, and an increased shelf life (Sanlier *et al.,* 2017). A number of previous studies showed that fermentation treatment on various plant-based raw feed materials could reduce the crude fiber content. Previous studies revealed that sago pulp mixed with the contents of the rumen (70:30) fermented with *Bacillus amyloliquefaciens* at a dose of 2% for nine days at 40°C could decrease its crude fiber by 33%, from 24.75% to 16.56%. Based on this background, the present study aimed to evaluate the fermentation method on sago pulp and its effect on the crude fiber content, digestibility, and utilization of sago pulp as a raw material for Nile tilapia *Oreochromis niloticus* feed.

MATERIALS AND METHOD

Fermentation of the sago pulp

The sago pulp used in the present study was waste obtained from a sago flour processing plant in Bogor, West Java. Wet sago pulp was sun dried for three days until the moisture content decreased to approximately 18%. The sago pulp was then ground into flour and sifted using a 75 µm (200 mesh) sieve.

The fermentation of the sago pulp was conducted by first steaming the sago pulp for 30 minutes and then cooling and weighing. The next step was adding the three fermentor treatments: tapai yeast, Baker's yeast, and sheep rumen fluid at different doses. Tapai yeast and Baker's yeast were added at different doses: 10 g/kg, 20 g/kg, 50 g/kg, 70 g/kg, and 100 g/kg, whereas the sheep rumen fluid was added at doses of 100 mL/kg, 200 mL/kg, 300 mL/kg, 500 mL/kg and 1000 mL/kg. The fermentors were then thoroughly mixed with the sago pulp flour and subsequently placed in

black plastic bags, tighthened with rubber bands and placed in canisters for an incubation period of 96 hours. After that, samples from each treatment were collected every 24 hours for crude fiber testing. The fermented sago pulp was then heated in an oven for 1–2 hours at 60°C. The fermentor which could decrease the greatest amount of crude fiber, tapai yeast at a dose of 50 g/kg incubated for 72 hours, was used as a reference for fermenting the sago pulp in the next stage (the digestibility test). Before and after the fermentation treatment, an analysis of the crude fiber and fiber fraction content were conducted on the sago pulp using the Van Soest method (1991).

Digestibility test

Experimental diet formulation

The second stage of the study was to evaluate the digestibility of the raw materials, i.e unfermented sago pulp flour and sago pulp flour fermented with tapai yeast at a dose of 50 g/kg for 72 hours. The research design in the digestibility test was a complete randomized design with 3 dietary treatments with 4 replications, i.e. reference diet (RD), unfermented sago pulp feed (AS), and fermented sago pulp feed (ASF).

The digestibility test was conducted based on Watanabe (1988) with a composition of 70% reference diet and 30% test material. The composition of the experimental diets used in the present study is presented in Table 1.

Fish maintenance and the collection of the digestibility test data

Seven Nile tilapia juveniles with an average weight of 25 g were kept in a 60x40x50 cm^3 aerated aquarium. The tested fish were adapted to the experimental diet for a week with a feeding frequency of three times a day at 08:00 AM, 1:00 PM, and 5:00 PM. After the adaptation period, the fish were fasted for 24 hours and then fed with the experimental diets and had their feces collected for 20 days from day three after the first day of experimental diet administration. The feed

Table 1. Composition and proximate composition of experimental diets in digestibility test

Feed composition (%)	Treatment feed		
	RD	AS	ASF
Fish meal	20	13.94	13.94
Soybean flour	24	16.73	16.73
Wheat flour	52	36.24	36.24
Palm oil	2	1.39	1.39
Sago pulp	-	30	-
Fermented sago pulp	-	-	30
Premix vit + min[1]	1	0.7	0.7
Binder	0.4	0.4	0.4
Cr_2O_3	0.6	0.6	0.6
Feed proximate (% dry weight)			
Protein (%)	33.40	26.39	27.26
Fat (%)	6.41	4.14	4.65
Ash (%)	8.26	8.98	8.37
Crude fiber (%)	3.68	9.22	6.93
NFE[2] (%)	48.25	51.27	52.79
GE[3] (kcal/kg)	4442	3817	4126

[1]Each gram of premix contained 50 IU of vitamin A, 10 IU of vitamin D, 0.60 IU of vitamin E, 50 mcg of vitamin K3, 100 mcg of vitamin B1, 200 mcg of vitamin B2, 650 mcg of vitamin B3, 300 mcg of vitamin B5, 0.25 mcg of vitamin B6, 5 mcg of vitamin B7, 40 mcg of vitamin B9, 0.250 of vitamin B12, 400 mcg of vitamin C, 2000 mcg of stay-c, 3000 mcg of α-tocopherol acetate, 1000 mcg of inositol, 3000 mcg of choline chloride, 280 mcg of phosphorus, 5600 mcg of potassium, 5600 mcg of calcium, 1820 mcg of magnesium, 8400 mcg of sodium, 196 mcg of iodine, 1.4 mcg of copper, 332 mcg of iron, 3.5 mcg of manganese, 33.6 mcg of zinc, 50 mcg of cobalt, 10 mcg of selenium, 3000 mcg of $CaHPO_4$, 1400 mcg of NaCl, and 500 mcg of phytase.

[2]NFE: Nitrogen-Free Extract.

[3]GE: Gross Energy, 1 g protein = 5.6 kcal, 1 g fat = 9.4 kcal, 1 g carbohydrate/NFE= 4.1 kcal (Watanabe,1998).

was given to apparent satiation with a frequency of three times a day (Watanabe, 1988). The feces were collected using a siphoning hose 30 minutes after feeding after the leftover feed was removed from the water. The feces was then collected and stored at -20°C until further analysis. The feces was then dried in an oven at 110˚C for 4–6 hours, prior to protein, carbohydrate, and energy content analyses. The measurement of Cr_2O_3 in the feces was conducted using a spectrophotometer with a 350 nm wavelength according to the procedure in Takeuchi (1988). The parameters observed in this digestibility test included:

$$ADC\ (\%) = [1- (\frac{NP}{NF} \times \frac{IP}{IF})]$$

Notes:
ADC = Feed digestibility coefficient
NP = Nutrient in feed (%)
NF = Nutrient in feces (%)
IF = Cr_2O_3 in feces (%)
IP = Cr_2O_3 in feed (%)

$$DE\ (kcal/100g) = Ep - [Ef \times (\frac{IP}{IF})]$$

Notes:
DE = Digested energy (kcal/100gr)
Ep = Energy in feed material (kcal/100gr)
Ef = Energy in feces (kcal/100gr)
IP = Cr_2O_3 in feed (%)
IF = Cr_2O_3 in feces (%)

$$ADC\ (\%) = [1- (\frac{NP}{NF} \times \frac{IP}{IF})]$$

Notes:
ADC = Feed carbohydrate/protein digestibility coefficient
NP = Feed carbohydrate/protein content (%)
NF = Feces carbohydrate/protein content (%)
IF = Cr_2O_3 in feces (%)
IP = Cr_2O_3 in feed (%)

Table 2. Composition and proximate composition of the experimental diets in growth test

Feed composition (%)	Treatment feed		
	G	AS	ASF
Fish meal	7	7	7
PBPM	21	20	20
Soybean flour	27	27	26
Bran	9	12.5	11.2
Wheat flour	10.5	8	10.3
Cassava flour	18	-	-
Sago pulp	-	18	-
Fermented sago pulp	-	-	18
Coconut oil	1.5	1.5	1.5
Corn oil	2	2	2
Premix vit + minerals	2	2	2
Binder	2	2	2
Proximate feed (% dry weight)			
Protein (%)	32.05	31.97	32.37
Fat (%)	5.51	5.71	5.51
Ash (%)	8.52	8.25	8.43
Crude fiber (%)	2.28	5.02	3.75
NFE (%)	35.49	32.48	33.56
GE (kcal/kg)	4098	3989	4037
C/P	12	12	12

Notes: G= 18% *gaplek*; AS= 18% sago pulp; ASF= 18% fermented sago pulp; NFE= Nitrogen-Free Extract; GE= gross energy, 1 g protein = 5.6 kcal, 1 g fat = 9.4 kcal, 1 g carbohydrate 4.1 kcal (Watanabe, 1988); PBPM= poultry by-product meal.

The digestibility rate of each test material used could be calculated using an equation suggested by Watanabe (1988):

$$\text{Material digestibility} = (\text{ADT} - 0.7\ \text{AD})/0.3$$

Notes:
ADT = test feed digestibility rate
AD = reference feed digestibility rate

Measurement of blood glucose content

The present study also conducted an observation of the blood glucose concentration before and after the administration of the tested diet. Before the blood collection, the fish were not fed for 24 hours. The blood was then collected at 0 hours (prior to feeding). The fish were then fed to satiation and blood was collected at 1, 2, 3, 4 and 5 hours post feeding. Blood samples were collected from the caudal vein using a 1.5 mL syringe that had been rinsed with 3.8% sodium citrate anticoagulant. One mL of the blood sample was placed in a 1.5 mL microtube and centrifuged at 3000 rpm for 10 minutes (Wedemeyer & Yasutake, 1977) for the measurement of blood plasma glucose and then analyzed using an ortho-toluidine reactant (Dubowski, 2008) and spectrophotometer at a wavelength of 530 nm.

Growth test

Experimental diets formulation

The growth test compared three types of experimental diets, i.e. diet with 18% cassava flour as the source of carbohydrate (G) as the comparative feed and feed with unfermented sago pulp flour (AS) and fermented sago pulp flour (ASF) as the source of carbohydrate, each 18% (Table 2). Cassava flour was used as the comparative material because this material is one of the carbohydrate sources commonly used in the production of fish feed in Indonesia (Henriksson *et al*., 2017).

Fish maintenance during the growth test

In the growth test, Nile tilapia juveniles with an average initial weight of 3.73 ± 0.08 g were kept in twelve units of 60×50×40 cm^3 aquariums with a water volume of 75 L. The fish were stocked at a density of 15 individuals per aquarium and were kept for 60 days. The feed was given to apparent satiation with a feeding frequency of three times a day at 08:00, 12:00, and 16:00.

Throughout the maintenance period, the water quality was maintained at an optimum range for the growth and survival of Nile tilapia by daily siphoning to remove any leftover feed and feces, and by replacing 50% of the water every 4 days. Each aquarium was equipped with a thermostat to maintain a stable temperature, aeration to regulate dissolved oxygen, and a top filter to reduce turbidity and ammonia concentration. The water temperature was measured daily in the morning, whereas the measurements of pH and dissolved oxygen were taken at the beginning, in the middle, and at the end of the maintenance period. Weight sampling was conducted three times during the course of the maintenance period.

At the beginning of the maintenance period, the fish's initial weight was recorded and 25 fish were collected as samples for the initial proximate analysis. At the end of the maintenance period, all the fish in every aquarium were weighed and 5 fish were collected for blood sampling for the blood profile observation (erythrocytes, leukocytes, and phagocytic activity) and then dissected to collect the livers for measuring the hepatosomatic index (HSI) and liver glycogen content. In addition, some of the samples were also used for the final fish proximate analysis as a basis for calculating the protein and fat retention.

Test parameters

The test parameters observed in this stage included the fish growth performance, the liver glycogen content, HSI, and protein and fat retention. The fish growth performance parameters included fish survival, specific growth rate, total feed intake, and feed conversion ratio. The specific growth rate (SGR) and feed conversion ratio (FCR) were calculated based on the equation suggested by Huisman (1987). The total feed intake (TFI) was determined by weighing the feed given during the maintenance period. The survival was calculated based on the proportion of fish that were still alive at the end of the maintenance period.

Extraction and hydrolysis of the liver were conducted based on the method by Watanabe (1988), while the liver glycogen analysis was conducted based on Dubowski (2008). The HSI value was calculated based on the ratio between the liver weight and the fish's total body weight. Protein retention and fat retention were calculated using a proximate analysis of the test fish's initial and final body protein and the fish feed protein. The equation used to calculate protein retention or fat retention is as follows (Takeuchi, 1988):

$$\text{RP/RL} = \frac{\text{F}-\text{I}}{\text{P}} \times 100$$

Notes:
RP/RL = Protein or fat retention (%)
F = The amount of the fish's protein/fat at the end of the maintenance period
I = The amount of the fish's protein/fat at the beginning of the maintenance period
P = The amount of protein/fat consumed

Proximate analysis of the feed and the fish

Proximate analyses were conducted on the feed raw material, experimental diets, and fish which consisted of the crude protein content which was conducted using the Kjeldahl method, the crude fat content which was conducted using the Soxhlet method, the fat content with the Folch method, the ash content by heating the samples in a furnace at 600°C, the crude fiber by dissolving the samples using strong acids and bases and heating them, and the moisture content by heating the samples in an oven at 105–110°C (Takeuchi, 1988).

Statistical analysis

The measurement data were calculated using Microsoft Excel. The crude fiber content data were analyzed descriptively, while the fiber fraction, digestibility, and growth performance data were analyzed statistically using the SPSS version 16.0 statistical software. For the fiber fraction and digestibility test, the ratio between unfermented and fermented sago pulp flour was obtained using the T-test at a confidence interval of 95%, whereas the growth performance data were analyzed using the ANOVA test at a confidence interval of 95%. Before the ANOVA test, the homogeneity of variances and data normality were tested using the Kolmogorov Smirnov test. If the ANOVA test gave a significantly different result, the analysis of variance between treatments was continued with Duncan's test.

RESULTS AND DISCUSSION

Results

The fermentation of sago pulp

The results of the first stage of the study which was the fermentation of sago pulp are presented in Table 3. The greatest decrease in crude fiber was seen in the treatment with tapai yeast at a dose of 50 g/kg and an incubation period of 72 hours, decreasing the crude fiber by 35.76%. On the other hand, the sheep rumen treatment at various doses and incubation periods did not demonstrate any effect on the sago pulp crude fiber content.

Table 3. The crude fiber % content of sago pulp flour fermented using three different fermentors with different doses and incubation periods

Fermentor	Dose	Incubation period (hours)				
		0	24	48	72	96
Tapai yeast	0 g/kg	18.76	18.76	18.76	18.76	18.76
	10 g/kg	18.56	18.45	17.80	16.00	15.00
	20 g/kg	19.11	18.45	16.20	14.40	13.23
	50 g/kg	18.76	18.45	15.38	12.05	13.12
	70 g/kg	19.00	18.88	16.10	12.23	13.09
	100 g/kg	19.22	18.20	16.55	13.42	13.18
Baker's yeast	0 g/kg	18.76	18.76	18.76	18.76	18.76
	10 g/kg	19.00	18.86	16.54	16.00	16.00
	20 g/kg	19.05	18.56	18.23	16.22	14.11
	50 g/kg	18.91	18.00	15.44	15.07	15.39
	70 g/kg	19.20	18.00	16.60	15.00	14.91
	100 g/kg	19.88	18.00	17.35	15.80	14.88
Sheep rumen fluid	0 mL/kg	18.76	18.76	18.76	18.76	18.76
	100 mL/kg	19.20	19.00	18.76	19.40	19.89
	200 mL/kg	18.68	18.70	18.00	19.00	18.00
	300 mL/kg	19.00	19.30	19.07	19.00	19.00
	500 mL/kg	19.00	18.30	18.00	18.00	18.00
	1000 mL/kg	19.21	19.22	18.60	18.17	19.72

Table 4. The proximate composition of unfermented sago pulp and after fermentation with tapai yeast at a dose of 50 g/kg for 72 hours.

Nutrient (% dry weight)	AS	ASF
Protein	1.72	6.23
Fat	0.5	0.49
Moisture content	15	13.7
Ash content	6	7.31
Crude fiber	18.76	12.05
NFE	58.02	60.22

Notes: AS= Sago pulp; ASF= Fermented sago pulp; NFE=Nitrogen-free extract.

The effects of fermentation using 50 g/kg tapai yeast at a 72 hour incubation period on changes in the sago pulp nutrients are presented in Table 4. The results of the protein content, fat content, ash content, crude fiber content, and nitrogen-free extract (NFE) analyses revealed that there was a 35.76% decrease in the sago pulp's crude fiber content from 18.76% to 12.05%, and protein content increased 2.6 times from 1.72% to 6.23%. On the other hand, the ash content, moisture content, fat content, and NFE demonstrated only little change.

Treatment by fermentation using 50 g/kg tapai yeast for 72 hours could decrease the neutral detergent fiber (NDF) fraction by 32.68% and hemicellulose by 60.39%, whereas the acid detergent fiber (ADF), cellulose, and lignin contents did not undergo any significant changes (Figure 1).

Digestibility test

The results of the digestibility test on sago pulp fermented with tapai yeast at a dose of 50 mg/kg and incubation period of 72 hours revealed that the fermentation of the material had a significant effect on the dry material digestibility and carbohydrate digestibility but not on the protein digestibility or energy digestibility (Figure 2).

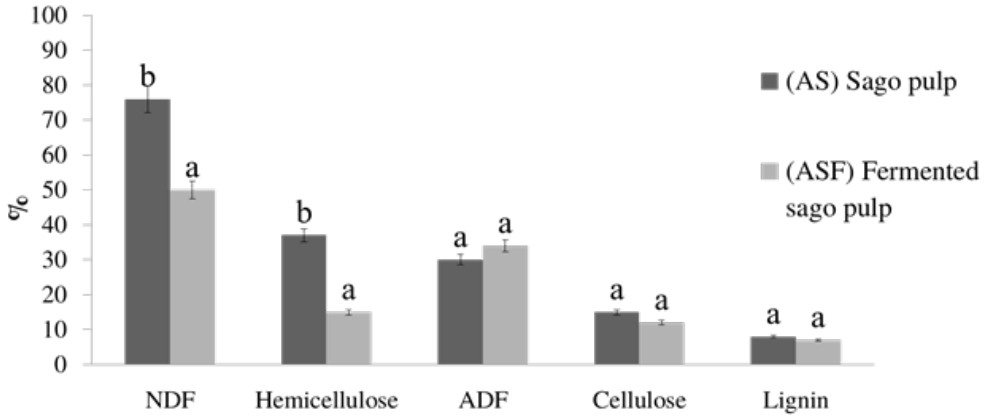

Figure 1. The fiber fraction contents of unfermented sago pulp (AS) and sago pulp fermented using 50 g/kg tapai yeast for 72 hours (ASF). Different letters above the bars indicate a significant difference (P<0.05).

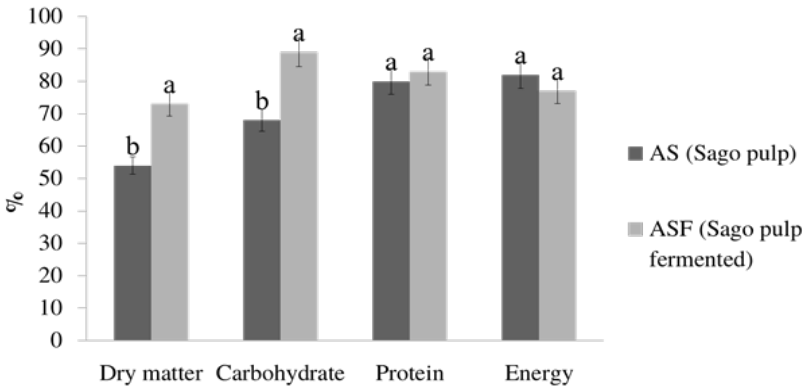

Figure 2. The digestibility of the material, carbohydrate, protein, and energy of sago pulp fermented with 50 g/kg tapai yeast for 72 hours.

The fermentation treatment could increase the dry material digestibility of sago pulp by 34% and the carbohydrate digestibility by 21%, but there were no significant changes in the protein digestibility or energy digestibility.

The result of the blood glucose content (Figure 3) demonstrated that at the 0 hours after the 24-hour fast the blood glucose of the Nile tilapia was below 40 mg/dL and started to increase in the 1st hour and reach a peak in the 2nd hour post treatment feed administration. The fermented sago pulp (ASF) treatment demonstrated the highest blood glucose at 134 ± 6.00 mg/dL during the 2nd hour. On the other hand, the highest blood glucose in the reference feed treatment and sago pulp (AS) treatment occurred in the 2nd hour and only reached a range of 120 ± 14.00 mg/dL and 94 ± 5.00 mg/dL, respectively. The blood glucose in the fish in all the treatments began to decrease from the 3rd to the 5th hour.

Growth assessment

The growth assessment that compared the feed with different carbohydrate source treatments (cassava flour, sago pulp, and fermented sago pulp) at 18% demonstrated different results between treatments (Table 5). The treatment using fermented sago pulp (ASF) demonstrated SGR, liver glycogen, fat retention, and protein retention values that were higher (P<0.05) than the treatment with sago pulp (AS), and were not significantly different from that with cassava flour (G) except for protein retention, which was higher in ASF compared to that of G treatment. Feed conversion ratio in ASF treatment was lower than that of AS treatment, but was comparable

Table 5. The initial biomass, final biomass, specific growth rate, feed conversion rate, protein retention, fat retention, hepatosomatic index, and liver glycogen of the Nile tilapia at the end of the study

Assessment parameter	Treatment (18% of carbohydrate source)		
	G	AS	ASF
Initial weight (g)	3.72 ± 0.09[a]	3.77 ± 0.74[a]	3.68 ± 0.57[a]
Final weight (g)	23.42 ± 2.90[a]	22.53 ± 2.36[a]	26.10 ± 1.98[a]
SGR (%/day)	3.10 ± 0.13[ab]	2.98 ± 0.18[b]	3.31 ± 0.12[a]
FCR	1.09 ± 0.05[a]	1.30 ± 0.05[b]	1.18 ± 0.10[ab]
Survival (%)	80.00 ± 21.08[a]	80.00 ± 12.17[a]	90.00 ± 3.84[a]
Protein retention (%)	35.26 ± 5.97[b]	31.58 ± 4.77[b]	47.34 ± 5.23[a]
Fat retention (%)	65.54 ± 22.48[ab]	47.80 ± 12.02[b]	85.58 ± 5.44[a]
HSI	2.12 ± 0.17[a]	1.72 ± 0.31[a]	2.06 ± 0.32[a]
Liver glycogen	0.12 ± 0.03[a]	0.06 ± 0.02[b]	0.10 ± 0.03[ab]

Notes: Different superscript letters after the average values (±standard deviation) in the same row indicate a significant difference (P<0.05). SGR= specific growth rate, FCR= feed conversion ratio, HSI= hepatosomatic index.

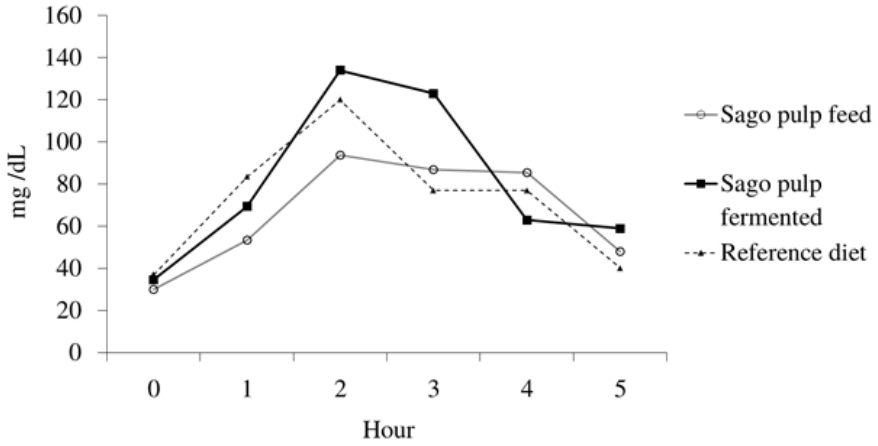

Figure 3. Changes in the blood glucose concentration of Nile tilapia after being given Sago pulp feed and sago pulp fermented with 50 g/kg tapai yeast for 72 hour.

to that of G treatment. The final weight, survival and HSI were not significantly different between treatments.

Discussion

The first stage of the study revealed that the type of fermentor had an effect on the decrease in sago pulp's crude fiber. The present study clearly demonstrated that the types of fermentors that could decrease crude fiber were tapai yeast and Baker's yeast. Yeast (both tapai and Baker's yeast) has been suggested to be able to perform well on sago pulp substrate. The sago pulp substrate provided the nutrients required for optimum growth of yeast and the fermentation process succeeded. The successful fermentation was proven by the change in the sago pulp substrate nutrient composition in particular the decrease in crude fiber by 35.76%. The results of the present study support a previous study by Sumardiono *et al*. (2018) where pre-treatment of sago pulp with 4% NaOH and fermentation with 1% *Trichoderma* sp for 14 days could decrease the crude fiber of sago pulp from 33.37% to 17.36% and increase the protein from 4.00% to 7.96%. In contrast with previous studies, treatment with rumen fluid did not have any effect on the crude fiber content of sago pulp flour. This is likely due to the incompatibility between the sago pulp substrate and the microorganisms and enzymes in the sheep rumen, causing the microorganisms and enzymes to demonstrate less than optimum performance. It is also possible that the presence of an inhibiting compound in the sago pulp that hindered the performance of the enzymes and microorganisms in the sheep rumen.

Tapai yeast contain a number of microorganisms from yeast and bacteria group such as *Bacillus* spp, *Rhizopus* sp, *Candida parapsilosis, C. melinii, C. lactosa, C. solani, Hansenula subpelliculosa, Rhizopus oligosporus, Aspergillus f1avus, A. oryzae,* and *Hansenula malanga* (Barus, 2013; Aslamyah, 2018). Yeast produces a number of extracellular enzymes such as amylase, amyloglucosidase, pectinase, cellulase, catalase, and glucosidase (Hardjo *et al*., 1989) which degrade the sago pulp substrate so that it could be utilized by the microorganisms to grow and develop. A few *Bacillus* spp produce enzymes that can hydrolyze oligosaccharide into easily digestible sugar (Ghani *et al.,* 2007). Specifically, *Rhizopus* sp in tapai yeast produces the amylolytic glucoamylase enzyme which can decrease amylose and amylopectin content by hydrolyzing the α-1.4 and α-1.6 glycoside link in the carbohydrate compound (Linggang *et al*., 2012). In addition, the microorganisms in the yeast can produce cellulase which degrades cellulose and hemicellulose into glucose (Ginting, 2018).

The different fermentor dose and incubation period treatments in the treatment with tapai yeast and Baker's yeast could decrease the crude fiber with a varied degree of decrease, but the greatest decrease was demonstrated by the tapai yeast treatment at a dose of 50 g/kg which was 35.76%. This suggested that the ratio between the amount of the fermentor and the amount of substrate and incubation period had an effect on the success of the fermentation process. The incubation length had an effect on the crude fiber content of the sago pulp. During the first 24 hours of the incubation, no changes were seen in the sago pulp's crude fiber content. This was probably because during this time period the microorganisms found in the yeast experienced a lag phase or adaptation period to a new environment. The decrease in crude fiber started to become apparent between the 48th to 72nd hour which was suggested to be the exponential phase where the fermentor microorganisms had actively degraded the fiber to a simpler material or compound. Meanwhile, during the 96th hour, the microorganisms began to enter the stationary phase where the growth of the microorganisms began to be limited by the availability of one or more nutrients or substrate. This was signified by the lack of any change in the crude fiber content in the sago pulp during the 96th hour. The results of the present study support a previous study (Santos, 2016) which demonstrated that *Aspergillus niger* and *Rhizopus* produced endoglucanase optimally after 70.35 hours at 29.56^{o}C and cellulase after 72.48 hours at 27.86^{o}C. Aslamsyah(2018) demonstrated that the use of a combination between 1.5% tapai yeast as a source of *Rhizophus* sp and 1.5% Baker's yeast as a source of *Saccharomyces* sp for the fermentation of the green strain *Kappaphycus alvarezii* seaweed flour for 72 hours could decrease the crude fiber by 28.60%, from 15.73% to 11.23%. Meanwhile, Kurniati (2017) reported that the optimum incubation of Jatropha seeds using a 0.3% fermentor dose (*Aspergillus niger* 0.15% and 0.15% *Rhizopus oryzae*) was 72 hours with the greatest decrease in crude fiber up to 21.36%. As for the fermentation of sago pulp, Ginting (2018) demonstrated that the fermentation of sago pulp using 1% "Ginta" local microorganism (MOL) up to 144 hours resulted

in an increased protein content in the sago pulp from 3.22% to 5.58% and a decrease in fiber content from 19.52% to 18.23%. The results of this particular study were still lower than the results of the present study which demonstrated the fermentation of sago pulp using tapai yeast at a dose of 50 g/kg and a shorter incubation, 72 hours, could decrease the crude fiber from 18.76% to 12.05% and increase the protein content from 1.72% to 6.23%. Another study by Sumardiono *et al.* (2018) demonstrated that immersion of sago pulp in a NaOH solution and fermentation using *Trichoderma* sp for 14 days could decrease the crude fiber content from 33% to 17% and increase the protein content from 4% to 8%. The comparison between the results above indicated that the success of the fermentation is influenced by the type of fermentor, the fermentor dose, and the incubation length.

The treatment by fermentation using tapai yeast at a dose of 50 g/kg and an incubation period of 72 hours appeared to have an effect on the fiber fraction content. Fermentation with this method resulted in a decrease in NDF by 32.68% and hemicellulose by 60.39%. However, the ADF, cellulose and lignin fiber fractions showed relatively no changes. A previous study by Yanti *et al*. (2014) revealed that fermentation of rice bran using *S. cerevisiae* and *A. niger* could decrease the NDF by 4.85% and hemicellulose by 40.98% and surprisingly increased the cellulose and lignin content. On the other hand, the study by Santoso *et al*. (2017) revealed that the fermentation of agricultural waste substrate using a combination of microbes (lactic acid bacteria, yeast, and cellulolytic bacteria) could decrease the ADF and NDF fiber fractions. The ADF fiber fraction is a fraction of fiber which includes cellulose and lignin from plant cell walls and some xylan and other components (Kramer *et al*., 2012). Moreover, Messana *et al.* (2013) stated that the enzymes produced by microbes could decrease the NDF level in substrates from various plants.

In the digestibility test, the fermentation treatment could improve both dry matter digestibility and carbohydrate digestibility of sago pulp. The improved ASF digestibility might relate to the decrease in the sago pulp's crude fiber after the fermentation treatment. The results of the present study demonstrated that crude fiber could be broken down into simpler materials so the NDF and hemicellulose fiber fraction decreased and sago pulp became more easily digested (Santoso *et al.,* 2017). The fermentation treatment could also change the chemical structure of the components of a certain material into simpler forms.

The improved digestibility of ASF would have a positive effect on nutrient absorption. This was seen in the result of the blood glucose measurements which revealed that the glucose absorption in the 2nd hour post feed administration in the fish treated with ASF was higher than that of fish treated with AS. The study by Aslamsyah (2018) demonstrated that the maximum absorption of glucose from the fermentation of the *Gracilaria gigas* seaweed occurred three hours post feeding. This result suggested that the use of fermented sago pulp could improve glucose absorption. Glucose is an energy source for animals that will be converted into a form of chemical energy (ATP) (Kamalam *et al.,* 2017). If the energy obtained from carbohydrate sources is adequate, the utilization of energy from other sources such as fat and protein would decrease, leading to retention of protein and fat in the body. This was proven by the significantly higher fat and protein retention value than that of the AS treatment.

The surplus of glucose originating from carbohydrate absorption which is not used as an energy source will be stored as glycogen or lipid in the liver (Li *et al*. 2016). In the present study, the liver glycogen content in the ASF treatment was not significantly different from that of the AS treatment. It was suggested that this could be because the amount of glucose contributed by the ASF feed could be utilized optimally as an energy source. In addition, the plasma glucose regulation in omnivorous fish such as Nile tilapia is known to be fairly efficient, which is evident in the fact that carbohydrate intake from feed could trigger glycolysis and lipogenesis and reduce gluconeogenesis, the synthesis of glucose from non-carbohydrate compounds (Qiang, 2013).

The availability of an energy source from carbohydrates could play a role in the protein-sparing effect, the utilization of energy from non-protein sources so protein could be utilized optimally for growth (Kamalam *et al*., 2017). Nile tilapia is an omnivorous fish that effectively utilizes carbohydrate, leading to more efficient utilization of feed protein sources in building the body (Boonanuntanasarn, 2018). The growth and protein-sparing factors are strongly related to the main function of carbohydrate as an oxidative substrate in muscle tissue and blood cells. The availability of carbohydrates in fish feed could

decrease gluconeogenesis, leading to a decrease in amino acid oxidation as an energy source and more optimum use of these amino acids in protein synthesis (Kamalam *et al*., 2017). The improved efficiency in protein utilization was demonstrated by the increased protein retention followed by an increased SGR and a decreased FCR in the treatment with ASF compared to those in the treatment with AS. Velasques *et al.* (2015) stated that utilization of up to 25% fermented duckweed in Nile tilapia had no adverse effect on the SGR or FCR, whereas the study conducted by Farizaldi (2017) demonstrated that the use of 20% fermented coconut pulp resulted in the highest growth rate, feed retention, and FCR in catfish. Virnanto *et al.* (2016) reported that the use of 20% fermented azolla flour (*Azolla microphylla*) resulted in the best growth and feed efficiency in giant gourami.

CONCLUSION

Fermentation using tapai yeast at a dose of 50 g/kg for 72 hours could decrease the crude fiber content and improve dry matter and carbohydrate digestibility of sago pulp so that it could be used as a source of carbohydrate in Nile tilapia feed.

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