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SOLID SUBSTRATE FERMENTATION OF SAGO WASTE AND ITS EVALUATION AS FEED INGREDIENT FOR RED HYBRID TILAPIA

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ABSTRACT

The increasing demand for fishmeal in aquaculture industry causes the rising cost of feed yearly. Here, we reported on the solid-state fermentation of sago waste inoculated with mixed microbial cultures, optimization of its fermentation parameters to improve the nutrient contents, and the use of the fermented sago waste as an ingredient in the formulation of fish feed diet. The use of *Bacillus amyloliquefaciens*, *Aspergillus niger*, and *Neurospora crassa* as inoculums gave the best, improved physiochemical properties and nutrient content of the fermented sago waste. The optimum conditions were 5 days, 28°C, pH 3, and sodium nitrate as a nitrogen source. Under the optimized conditions, moisture content, crude protein, and ash increases by 11.8%, 1.3%, and 5.1%; whereas dry matter and crude fiber decreases by 11.8% and 6.1%; respectively. The fermented sago waste prepared was further used as ingredient in the formulation of the fish feed diet and fed to red hybrid tilapia. Fish feed that contained up to 150 g kg^{-1} of fermented sago waste had a similar growth rate. Growth performance, specific growth rate, feed conversion ratio, and survival rate of tilapia fed were not of significant difference compared to control diet. The supplementation of the diets for 60 days resulted in 1.50 ± 0.48 g $fish^{-1}$ mean body weight gain with a specific growth rate of 0.68 ± 0.17% day^{-1}, feed conversion ratio of 2.33 ± 0.84, and survival rate of 80%. This concludes that fermented sago waste has the potential as a partial substitute for fishmeal.

Key words: *Aspergillus niger*, *Bacillus amyloliquefaciens*, mixed culture, *Neurospora crassa*, nutrient enhancement, Sago waste, solid-state fermentation

INTRODUCTION

Our environments are broadly exposed to pollution by agro-industrial by-products (Ali *et al*., 2011; Sarkar *et al*., 2012; Soliman *et al*., 2013; Anwar *et al*., 2014; Basappaji & Nagesha, 2014). According to Tan and Li (2017), global funding to solve the environmental issue regarding waste management had increased. Malaysia is the world's largest sago (*Metroxylon sagu*) starch exporter with 96% of starch production that came from the state of Sarawak (Uthumporn *et al*., 2014).

Residues from sago starch processing mills such as sago waste are often deposited as waste and reported to cause environmental problems (Bujang *et al*., 1996; Awg-Adeni *et al*., 2013; Lim *et al*., 2019). Awg-Adeni *et al.* (2013) stated that Sarawak produced approximately 7.1 tons (t) of starchy fibrous sago pith waste daily from a single sago starch-processing mill. The sago agro-industrial waste consists mainly of non-starch polysaccharides (NSP) or lignocellulosic materials such as cellulose, hemicellulose, and lignin (Lai *et al*., 2013). Lignocellulosic waste has many potentials to be converted to value-added products. Sago waste has the potential to be utilized as a carbon source in the production of valuable products (Vincent *et al*., 2015; Lim *et al*., 2019).

High fiber and low protein content may reduce the quality of sago waste to be accepted as aquaculture feed (Awg-Adeni *et al*., 2010). It is suggested that the pre-treatment of lignocellulosic biomass using microbes can be beneficial in the production of byproducts and often of high quality

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(Maurya *et al*., 2015; Jönsson & Martín, 2016). Bacteria and fungi produce a wide range of highly active plant fiber degrading and hydrolytic enzymes.

In comparison to the nutritional values of fishmeal, different animal, plant, and microorganism-based sources have been studied extensively to replace fishmeal (Li *et al*., 2013). Fungal biomass cultured on various types of low-valued ligno-cellulosic waste can be one of the most applicable ingredients as fish feed (Zhang *et al*., 2013). Fermented lignocellulose and filamentous fungi, with high nutritional values such as protein content, important amino acids, immunostimulants, pigments, and antioxidants can also be used as an alternative supplement to fishmeal.

Several studies have reported that lignocellulosic agro-industrial by-products such as date fiber (Belal *et al*., 2015), rice bran (Hung *et al*., 2015), and palm kernel meal (Adjanke *et al*., 2016) to name a few can be used as aquaculture feed. Therefore, sago lignocellulose waste was treated using cellulase-, xylanase- and mannanase-producing microbes to further improve its nutritional quality as animal feed (Li *et al*., 2013; Lim *et al*., 2019; Rasyid *et al*., 2020).

Solid-state fermentation (SSF) has emerged as a potential technology to produce microbial products such as feed, fuel, food, industrial chemicals, and pharmaceutical products (Mussatto *et al*., 2012; Vincent *et al*., 2015). It can be defined as the fermentation process that occurs in the absence or near-absence of free water and generally employs a natural raw material as the carbon and energy sources (Soccol *et al*., 2017). Solid-state fermentation (SSF) is the method of choice as it stimulates the natural environment of most microorganisms especially fungi (Lizardi-Jiménez & Hernández-Martínez, 2017). Recently, SSF contributes more to the production of industrial enzymes, biopolymers, pigments, secondary metabolites, etc. (Robinson *et al*., 2001; Marzo *et al*., 2019).

As for the sago waste, solid-state fermentation (SSF) was mostly studied for enzyme production, animal feed, and bioethanol (Vincent *et al*., 2015; Elaiyaraja *et al*., 2016; Santoso *et al*., 2017; Hung *et al*., 2018). However, not much research done on SSF of sago waste using mixed cultures of bacteria and fungi and have yet to be investigated for fish feed (Liu *et al*., 2017; Sumardiono *et al*., 2018). The advantage of SSF is that it exhibits great potential in producing important enzymes that capable to break down the fibers thus improve digestibility. Furthermore, the SSF of lignocellulose biomass has also been used to improve the total protein content and other desired nutritional values (Li *et al*., 2013; Zhang *et al*., 2013; Jalil *et al*., 2015).

In the aquaculture industry, feed supply depends on common ingredients such as fishmeal, soybean meal, corn, wheat, rice, and fish oil. These common ingredients were also shared by other agricultural sectors such as for human direct consumption and animal husbandry in which leading to high competition between those sectors. Most of the key ingredients used in feed processing industries, which are the traditional recipes of farm aquaculture feed as well as for commercial use, are imported commodities. Given the rising prices of feedstuff and its ingredients constitute a real constraint for the development of aquaculture such as nutritional knowledge and identification of alternative feed ingredients (Shamshak & Anderson, 2010; Karimi *et al*., 2018).

Starting from the 1920s, the aquaculture industry remains an important sector of Malaysia's economy contributing 1.73% to the national GDP and providing employment for 16% of the population (FAO, 2004; FAO, 2018). Two main farming systems applied in the aquaculture industry in Malaysia are brackish water and freshwater aquaculture. The main species for brackish water are cockles, shrimps, tiger prawns, and marine fishes while freshwater is dominated by tilapia, catfish, and carps. In a developing country like Malaysia, the aquaculture industry faced problems such as rising production costs, lack of skilled labor, the threat of diseases, food safety, and quality of aquaculture produce (Mamauag, 2016).

One of the important production costs in the aquaculture industry is the production of its feed where it is always comprising within the range of 30% – 60% of the total production costs (Azaza *et al*., 2009). Also, the main ingredient for aquaculture feed, which is fishmeal, competes with the food of other agricultural sectors. As the aquaculture industry progresses, the demand for fishmeal as fish feed will always increase and the same goes for its prices. Therefore, to move toward a more sustainable aquaculture industry, it is essential to seek a renewable and cheap supplementation source that may partially or even totally replaces fishmeal (Hua *et al*., 2019).

With regards to sago waste, the disadvantage of using sago waste as a fish feed ingredient is due to its high crude fiber content which could inhibit the growth of the fish. One of the ways to overcome this problem is to conduct fermentation using microbes on sago waste. Previous studies conducted by Jalil *et al*. (2015) reported that solid-state fermentation using *Rhizophus oligosporus* improved the nutritive value of ground sago pith (GSP) by increasing the content of reducing sugar, glucose, and fructose, soluble protein, and amino acids in particular cysteine.

We hypothesized that solid-state fermentation of sago waste by fibrolytic and lignocellulolytic microbes will degrade fibrous materials, and at the same time, increases the protein content, thus

improve the overall nutritive values of fermented sago waste. In doing so, the appropriate microbial mixed cultures as inoculums, optimum fermentation parameters, and proximate composition of fermented sago waste were investigated. The process of SSF of sago waste was further upscale under the optimal experimental conditions for the preparation of the fermented sago waste and applied as a fish feed ingredient. Therefore, this research was aimed to study the production and application of fermented sago waste with enhanced nutritive values via solid-state fermentation as an alternative ingredient to fishmeal in the fish feed diet.

MATERIALS AND METHODS

Solid-state fermentation (SSF) of sago waste

Sago waste was kindly provided by Mukah Sago Plantation, in Mukah Division, Sarawak. The sago waste was air-dried, grounded, and sieved through a 2.0 mm sieve and stored in closed airtight containers at room temperature. The microorganisms used as inoculums in SSF were obtained from Molecular Genetic Laboratory, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak (UNIMAS) Microbial Collection.

Solid-state fermentation was carried out under sterilized conditions in 250 mL conical flasks that contained 10 g of sago waste as substrate. The initial moisture content of the culture medium was adjusted to 75% (w/w) (Kashyap *et al*., 2003). A volume of 20% (v/v) of minimal salt medium (MSM) was added to each flask for growth medium supplementation. Approximately, 20% (w/v) of microbial inoculum (dry weight basis) was added in combination (Table 1) to the sago waste substrate.

The content in the flasks was mixed well using a sterile spatula and all of the flasks were plugged with cotton and covered with aluminum foil. There were four sets of treatments carried out in three replicates each. In all the experiments the size of the inoculum was fixed at 20% (w/v) on a dry weight basis. The flasks were incubated at ambient temperature (28 ± 32°C) for 7 days. Unfermented sago waste served as control.

Table 1. Types of microbes used in solid-state fermentation (SSF) process

(Microbial strains) Type of Treatment	*BA*	*BL*	*AN*	*AF*	*NC*
Treatment 1	√	√	√	√	
Treatment 2	√		√		
Treatment 3	√			√	
Treatment 4	√		√		√

Notes: BA= Bacillus amyloliquefaciens, AN= Aspergillus niger, AF= A. flavus, BL= B. licheniformis, NC= Neurospora crassa.

The sago waste that was fermented with the best combination of mixed cultures that enhanced the nutritive value will be selected further to determine the optimum physiochemical parameters in solid-state fermentation (SSF) of sago waste. Four different fermentation physiochemical parameters investigated were period of fermentation, pH, temperature, and nitrogen source.

The large-scale solid-state fermentation of sago waste using mixed culture was later conducted to investigate the differences between nutrient enhancement in fermented sago waste between small scale (10 g sago waste) and large scale (250 g sago waste) under optimal fermentation experimental conditions.

Nutritional analysis of fermented sago waste

The proximate composition is the term usually used in the field of feed and means the six components of moisture, crude protein, ether extract (crude fat), crude fiber, crude ash, and nitrogen-free extracts which are expressed as the content (%) in the feed, respectively. The method used to analyze the proximate composition of the dried samples of unfermented and fermented sago waste was done according to the method as described by Horwitz (2000).

To determine the dry matter content, 2 g of the samples were dried in an oven at 105°C until constant weight. The samples were weighed before and after drying and the contents of dry matter were calculated. The crude protein content was determined by the Kjeldahl method as described by the AOAC method (Horwitz, 2000). The amount of protein was calculated by converting the amount of nitrogen by a factor of 6.25. The fat content was determined gravimetrically after extracting 1 g samples with diethyl ether (boiling point 35–38°C) in a Soxhlet Extractor for about 6 hr. The ash content was determined by combustion of 1.5 g samples in silica crucibles in a muffle furnace for 24 hr at 550°C. All the measurements were performed at least in triplicate and the data were expressed as means ± standard deviations.

Dietary feed formulation

Four isonitrogenous (30% ± 2.0 crude protein) experimental diets were formulated. The formulation of the diet is shown in Table 2. The diets prepared were as followed: all feed ingredients were ground in a commercial blender and mixed homogenously. The homogenous mixture of feed ingredients was mixed by hand. Vitamin and mineral mixes were gradually added with continuous mixing. Warmed distilled water (60°C) was slowly added while mixing until the mixture began to clump. Then, the diet was

Table 2. Formulation of the fish feed composition

Ingredients (g kg^{-1})	Diets (g 100^{-1} g dry matter)			
	0 g $100\ g^{-1}$	5 g $100\ g^{-1}$	10 g $100\ g^{-1}$	15 g $100\ g^{-1}$
Fish meal (70 g kg^{-1} CP)	30.00	29.00	28.00	27.00
Wheat bran	60.00	56.00	52.00	48.00
Fermented sago waste	0.00	5.00	10.00	15.00
Vitamin	2.00	2.00	2.00	2.00
Mineral mixes1	2.00	2.00	2.00	2.00
Wheat flour	5.00	5.00	5.00	5.00
Sunflower oil	1.00	1.00	1.00	1.00
Total	100	100	100	100

dried for 24 hr at 70°C in a vacuum drying oven. The dried diet was crumbled into small size and stored at room temperature in sealed plastic bags.

Fish culture condition and experimental design

Red hybrid tilapia fingerlings (3.03 ± 0.91 of average initial weights) were bought from Aquafish shop in Kuching, Sarawak. Fifteen fishes were stocked into 2 L tanks in a closed, recirculating indoor system. The tanks were provided with central drainage pipes surrounded by outer pipes, perforated at the bottom, to facilitate self-cleaning and waste removal. The fish culture system was provided with a biological filter, aeration through an air blower, and heaters to maintain the water temperature at 27°C. Approximately 10% (v/v) of the water volume was replaced by new freshwater daily. Lighting in the culture unit was set at 12:12; L: D cycle.

A fixed feeding regime of 4% of the bodyweight per day (dry food/whole fish) was employed for the first seven weeks and 3% of the bodyweight until the end of the experimental period. Fish were fed twice a day (10:00 and 17:00 hr) in equal portions for 60 days. Fish were fed for thirteen consecutive days, weighed on the fourteenth day, and feeding rates for the following week adjusted accordingly. Each diet was fed to triplicate groups of fifteen fish each to satiation level (satisfactory level of eating or unable to take on more). Fish were weighed collectively at fourteen-day intervals, their average weights and lengths were recorded.

Red hybrid tilapia growth performance analysis

Every two weeks' interval fish were bulk weighed for one aquarium at a time without anesthesia and the fish was weighed. The fish length was also recorded. Mortality and feed intake were monitored and recorded daily.

Feed efficiency performances including fish weight gain (WG) (Equation 1), specific growth rate (SGR) (Equation 2), and feed conversion ratio (FCR) (Equation 3) were calculated as follows:

$$\text{Weight Gain, WG} = W_2 - W_1,$$

where WG is the mean of weight gain, W_2 is the mean final weight, W_1 is the mean initial weight.

$$\text{Specific Growth Rate, SGR} = \frac{1n\ W_2 - 1n\ W_1}{\text{time in days}} \times 100$$

$$\text{Feed Conversion Ratio FCR} = \frac{\text{feed (dry) intake (g)}}{\text{wet weight gain (g)}}$$

Statistical analysis

Statistical analyses were performed using SPSS (version 13). Significant differences were evaluated with a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. A value of $p<0.05$ was considered significant.

RESULTS

Solid substrate fermentation of sago waste

To determine the optimal culture combination that able to enhance the nutrient in sago waste, solid substrate fermentation (SSF) of sago waste employing the microbial culture formulation was performed. The nutrient enhancement parameter in this study was based on the proximate analysis of the fermented sago waste composition.

The result of the proximate composition of fermented sago waste was summarized in Table 3. From four different culture combinations and one control, there is at least one culture that showed a statistically significant difference ($p<0.05$) in every composition tested for fermented sago waste. As expected, the moisture content, crude protein, and ash content in fermented sago waste showed a higher result as compared to the control, the unfermented sago waste while dry matter content and fiber content showed the lower result as

Table 3. Proximate composition of unfermented and fermented sago waste (%)

Culture	Moisture content (%)	Dry matter (%)	Crude protein (%)	Crude fiber (%)	Ash (%)
Untreated (Control)	$34.38 \pm 1.93_{a}$	$65.62 \pm 1.93_{a}$	$1.00 \pm 0.0_{a}$	$25.96 \pm 0.15_{a}$	$2.60 \pm 0.11_{a}$
BA + AN	$41.81 \pm 1.78_{ab}$	$58.19 \pm 1.78_{ab}$	$1.47 \pm 0.06_{b}$	$23.90 \pm 0.68_{b}$	$2.60 \pm 0.35_{a}$
BA + AF	$40.30 \pm 9.11_{ab}$	$59.70 \pm 9.11_{ab}$	$1.35 \pm 0.13_{b}$	$20.86 \pm 0.84_{c}$	$3.10 \pm 0.15_{a}$
BA + AN + NC	$46.22 \pm 1.95_{ab}$	$53.78 \pm 1.95_{ab}$	$2.28 \pm 0.03_{c}$	$19.85 \pm 0.28_{c}$	$7.70 \pm 0.31_{b}$
BA + BL + AN + AF	$48.44 \pm 3.06_{b}$	$51.56 \pm 3.06_{b}$	$1.33 \pm 0.06_{b}$	$23.75 \pm 0.60_{b}$	$2.80 \pm 0.06_{a}$

Notes: BA= *Bacillus amyloliquefaciens*, AN= *Aspergillus niger*, AF= *A. flavus*, BL= *B. licheniformis*, NC= *Neurospora crassa*. Each value is expressed as mean ± SD. The values in each row with the same letter are not significantly different at the level of 0.05 ($p>0.05$).

compared to the control. The data obtained indicated that mixed microbial cultures fermentation had enhanced the nutrient values in the fermented sago waste.

On the moisture and dry matter content, the sago waste fermented with a mixed microbial culture combination of *Bacillus amyloliquefaciens*, *B. licheniformis*, *Aspergillus niger*, and *A. flavus* showed a significant result as compared to the control and the other microbial cultures combination. The microbial culture with the combination of *B. amyloliquefaciens*, *A. niger*, and *Neuspora crassa* showed a significant increase in crude protein and ash content of fermented sago waste. Significant ($p<0.05$) decreases were also recorded in the crude fiber content of sago waste fermented with a mixed microbial culture of *B. amyloliquefaciens*, *A. niger*, and *N. crassa* as well as in sago waste fermented with a mixed microbial culture of *B. amyloliquefaciens* and *A. flavus*.

Optimization of solid substrate fermentation parameters

The sago waste was fermented with the optimal combination of mixed cultures that enhanced the nutritive value, which was the mixed microbial cultures of *B. amyloliquefaciens*, *A. niger*, and *N. crassa*, was further selected to determine the optimum physiochemical parameters in solid-state fermentation (SSF) of sago waste. Soluble protein content was analyzed using the Bradford method and crude protein using the standard Kjeldahl method. Four different fermentation parameters were investigated which were period of fermentation, pH, temperature, and nitrogen source. Other parameters of fermentation except for the optimized parameter were maintained as the same. All the experiments were carried out in duplicates and the average of the duplicates reading was presented. Figure 1 below shows the crude protein content of fermented sago waste at different levels of solid substrate fermentation physiochemical parameters tested.

When sago waste was inoculated with a mixed culture of *B. amyloliquefaciens*, *A. niger*, and *N. crassa*, at different pH ranged from pH 3.0 to pH 7.0, the highest crude protein content recorded was 1.84% at pH 3.0. From the statistical analysis done, only crude protein content at pH 3.0 shows a significant difference and this indicates that the optimum pH is pH 3.0 for mixed fermentation of sago waste with *B. amyloliquefaciens*, *A. niger*, and *N. crassa*. Among four levels of temperatures tested in this study, ranging from 28°C to 40°C, crude protein content at a temperature of 28°C, 36°C, and 40°C show no significant difference but only crude protein at a temperature of 30°C shows a significant difference. However, the best yield of protein content was recorded at a temperature of 28°C, 36°C, and 40°C which means that the optimum temperature of the SSF ranged from 28°C to 40°C.

The incubation period ranged from 5 days to 14 days showed no significant differences between crude protein content from 5 days to 7 days. However, significant differences showed by crude protein content at 10 days of the incubation period. This result shows that the optimum incubation period for the mixed culture of *B. amyloliquefaciens*, *A. niger*, *and N. crassa*, was ranged from 5 days to 7 days. As for nitrogen supplements, no significant differences were observed in crude protein content produced among four types of nitrogen supplement tested in this study. It shows that mixed culture of *B. amyloliquefaciens*, *A. niger*, and *N. crassa*, could produce optimum crude protein using any of the four types of nitrogen supplement tested.

Large-scale solid-state fermentation on sago waste composition under optimized condition

A large-scale solid-state fermentation of sago waste using a mixed culture of *B. amyloliquefaciens*, *A. niger*, and *N. crassa*, was conducted to investigate the differences between nutrient enhancement in sago waste between small scale (10 g sago waste) and larger scale (250 g sago waste). As predicted, the scaling up of the volume of the substrate in the solid-state fermentation of sago waste did not give any significant effect on the nutrient enhancement in sago waste as compared to the small-scale SSF. All the experiments were carried out in duplicates and averages of the duplicates reading were presented.

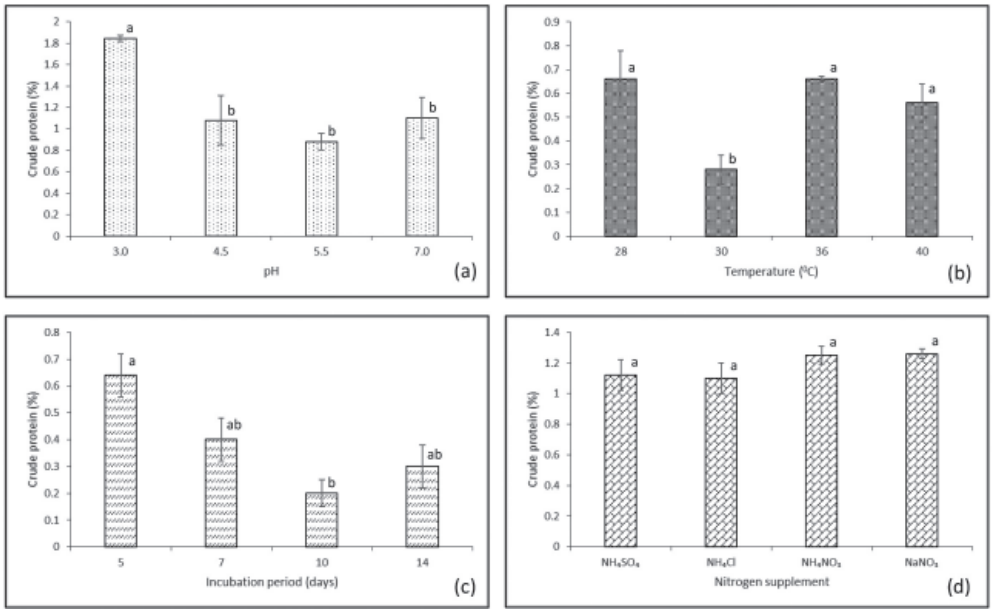

Fig. 1. Crude protein of fermented sago waste with the mixed microbial culture combination of *Bacillus amyloliquefaciens*, *Aspergillus niger*, and *Neurospora crassa*, at different (a) pH, (b) temperature, (c) incubation period, and (d) nitrogen source for SSF. The values in each column with the same letter are not significantly different at the level of 0.05 (p>0.05).

Table 4. Chemical composition of sago waste in small scale and larger-scale solid substrate fermentation

Combination of Cultures	Chemical Composition %				
	MC	DM	CP	CF	Ash
BA + AN + NC (10 g)	$46.2 \pm 0.49_a$	$53.8 \pm 2.12_a$	$2.3 \pm 0.28_a$	$19.9 \pm 0.57_a$	$7.7 \pm 0.42_a$
BA + AN + NC (250 g)	$47.9 \pm 0.91_a$	$52.1 \pm 1.06_a$	$2.3 \pm 0.14_a$	$31 \pm 2.55_b$	$5.1 \pm 0.21_b$

Notes: MC= moisture content, DM= dry matter, CP= crude protein, CF= crude fiber, BA= *Bacillus amyloliquefaciens*, AN= *Aspergillus niger* and NC= *Neurospora crassa*. Each value is expressed as mean ± SD. The values in each row with the same letter are not significantly different at the level of 0.05 (p>0.05).

The chemical composition of the fermented sago waste in small-scale and large-scale solid-state fermentation of sago waste was as presented in Table 4.

No significant differences were found in terms of moisture content, dry matter, and crude protein between SSF of 10 g and 250 g sago waste. However, the crude fiber and ash content showed a significant difference between 10 g SSF and 250 g SSF of sago waste. The crude fiber in 250 g SSF of sago waste remained higher by 11.1% as compared to crude fiber in 10 g SSF. The ash content in 250 g SSF of sago waste significantly decreases by 2.6%. The fermented sago waste was further used as fish feed diet formulation and ingredient in the subsequent fish feeding trial experiments.

Effects of feeding fermented sago waste on growth performance of red hybrid tilapia

Generally, the red tilapia fed with the experimental diets was observed in good condition, in terms of its growth performance, health and survival rate. Average values of initial weight, final body weight, weight and length gain, specific growth rate, feed conversion ratio, and survival rates of growing tilapia fed with different experimental diets supplemented with sago waste were presented in Table 5. The results indicated that there were no significant differences (p>0.005) among all treatments in initial weight which reflects homogeneity in fish weight at the beginning of the experiment.

No significant difference was observed in the final weight as revealed by the results between the control diet and each of the tested experimental diets. The result shows that the final mean weight gains were in the range from 4.2 g to 4.7 g which indicated that the weight gain of red hybrid tilapia in these eight weeks of feeding trial was increased approximately by 50% from its initial weight.

The highest final weight gain of 4.69 g was recorded in the experimental diet supplemented with 10% (w/w) of fermented sago waste, followed by 4.59 g experimental diet supplemented with 15% (w/w) of fermented sago waste of, 4.38 g experimental diet

Table 5. Performance of red hybrid tilapia fed with fermented sago waste-based experimental diets

Growth parameters	Experimental diets			
	Control	5%	10%	15%
Initial mean body weight (g fish^{-1})	2.91 ± 0.68	2.79 ± 0.74	3.33 ± 1.43	3.09 ± 0.79
Final mean body weight (g fish^{-1})	4.38 ± 1.38	4.23 ± 1.20	4.83 ± 2.67	4.59 ± 1.11
Mean body weight gain (g fish^{-1})	1.47 ± 0.70	1.43 ± 0.61	1.45 ± 1.27	1.50 ± 0.48
Initial body length (cm)	5.30 ± 0.49	5.20 ± 0.38	5.50 ± 0.69	5.40 ± 0.42
Final body length (cm)	6.10 ± 0.83	6.00 ± 0.82	6.40 ± 1.15	6.30 ± 0.59
Mean body length (cm)	0.87 ± 0.32	0.83 ± 0.47	0.90 ± 0.46	0.87 ± 0.15
Specific growth rate (% day^{-1})	0.65 ± 0.18	0.68 ± 0.17	0.58 ± 0.29	0.67 ± 0.16
Feed conversion ratio	2.62 ± 1.17	2.90 ± 1.00	3.48 ± 0.31	2.33 ± 0.84
Survival rate (%)	73.00 ± 2.65	80.00 ± 2.08	80.00 ± 6.35	73.00 ± 3.00

Notes: Values represent the means of three replicates. Each value is expressed as mean ± standard deviation (SD).

with no fermented sago waste (control) and the lowest final weight of 4.38 g was recorded in the experimental diet supplemented with 5% (w/w) fermented sago waste. All the values of the final weight gain were found almost similar and did not differ significantly (p>0.05).

The final body length of the experimental diet of control, 10% and 15% (w/v) sago waste were recorded similar to 0.9 cm and the experimental diet with 5% (w/w) fermented sago waste shows slightly lower with a final length of 0.8 cm. Specific growth rates feed conversion ratios and survival rates of all tested experimental diets also gave almost similar results with no significant differences among the control diet and other tested experimental diets.

Generally, the highest value for specific growth rate was obtained from a diet supplemented with 5% (w/w) fermented sago waste of 0.68% day^{-1}, and the optimal feed conversion ratio of 3.48 was recorded in the diet supplemented with 10% (w/w) fermented sago waste. Similar survival rates were recorded in 5% (w/w) and 10% (w/w) experimental diets of 80% followed by control and 15% (w/w) diets of 73% with no significant differences between them.

DISCUSSION

To date, no published research is done so far on the use of mixed microbial culture fermentation on sago waste in enhancing its nutrient value. The present study aimed to enhance the nutrient values in fermented sago waste using a different combination of selected mixed microbial cultures as inoculums under the solid-state fermentation of sago waste. Among the four combinations of microbial cultures tested in this study, the optimum combination of microbial cultures that enhanced the nutrient value of fermented sago waste was the mixed microbial cultures of *B. amyloliquefaciens*, *A. niger*, and *N. crassa*. Crude protein and ash content of sago waste treated with this microbial inoculums' combination increased significantly and fiber content decreased significantly as compared to other combinations of culture.

Combining single *Aspergillus* sp. with single *Bacillus* sp. either with *A. flavus* or *A. niger* gave insignificant differences in protein, ash, and fiber degradation. Using the two types of *Aspergillus* sp. and two types of *Bacillus* sp. also gave no significant difference in terms of nutrient enhancement of the fermented sago waste. However, the addition of *N. crassa* shows a significant result in nutrient enhancement of fermented sago waste. This agrees with the findings of Lio and Wang (2012) who discovered that inoculation of different types of fungi is the key factor in achieving higher production and better growth in solid-state fermentation. A significant decrease in fiber content of sago waste with this microbial culture combination confirmed the ability of *N. crassa* to degrade crude fiber as reported by Liu *et al*. (2016). The microfungi, *N. crassa* is also known as the potent producer of lignocellulolytic and hemicellulolytic enzymes (Sygmund *et al*., 2012).

In the optimization experiment of SSF of sago waste, pH 3.0 and 5 days of incubation period gave a significant maximum yield of protein enrichment in sago waste inoculated with *B. amyloliquefaciens*, *A. niger*, and *N. crassa*. These results agreed with other results, where Rauf *et al*. (2010) reported that the optimal pH condition for protease production in SSF of sunflower meal is pH 3.0. The pH is a critical factor in SSF and only adjusted at the beginning of SSF. It is hard to control throughout the process of SSF and its optimum point is depending on its SSF condition (Yoon *et al*., 2014). Shi *et al*. (2015) found that the optimal period of incubation for crude protein production in SSF of rapeseed cake using *A. niger* was 5 days. However, the result on the temperatures and nitrogen sources gave an insignificant result which indicated that the range of maximum yield of protein enrichment falls on temperatures of 28°C to 40°C and with any of the four different nitrogen sources.

Research done by Lio and Wang (2012) found that enzymes (xylanase and cellulase) production from the large scale SSF (300 g soybean fiber) were much lower than small scale (40 g soybean fiber) was consistent with the result of fiber degradation of this research in large scale (250 g sago waste) which lower than small scale (10 g sago waste) of fiber degradation. This result might be caused by large-scale SSF which limiting the potential of fungal growth and hyphae penetration in compact structures (Lio & Wang, 2012).

A variety of alternate resources have been studied to partially replace fishmeal in fish feed ingredients. Very little information is currently available on the use of fermented sago waste in fish diets. A previous study utilizing sago pith with rumen content for poultry feed (Wizna *et al*., 2008). Closer observation to the result of this study indicated that the present findings are consistent with the findings of other studies, in which, the result of experimental diets reached up to 20% inclusion of agroindustry by-products were not significantly different in terms of growth performance (p<0.05) when compared with the results of the control. However, there was a slow growth rate of fish fed with sago waste as compared to palm kernel meal and date fiber. This result might be due to other factors such as the quality of the culture system. According to Mota *et al*. (2019), the fish culture conditions might affect the result of fish weight and growth rates.

Ng and Chong (2002) have studied the growth performance of red hybrid tilapia fed with improved nutritive quality of palm kernel meal (PKM) included in the experimental diets. They found out that up to 20% inclusion of palm kernel meal gave no significant effects on fish growth performance in comparison to control diet without PKM. Belal *et al*. (2015) have evaluated the use of date fiber in feed ingredients of Nile tilapia, and they revealed that with up to 20% inclusion of date fiber, no significant effects were found on the growth of the fish. The present study shows that even though the nutrient content of fishmeal is better than the fermented sago waste, none of these differences were statistically significant (p>0.005) in red hybrid tilapia that were fed with fermented sago waste at 5%, 10%, and 15%, either in their feed conversion ratio or growth parameters.

CONCLUSIONS

The mixed microbial culture combination of *B. amyloliquefaciens*, *A. niger*, and *N. crassa* used as inoculums in the solid-state fermentation of sago waste can enhance the nutritive values of the fermented sago waste. Optimum SSF conditions for crude protein production in sago waste using the mixed microbial cultures combination are pH 3.0, 5 days incubation period with temperature ranging from 28°C to 40°C, and able to use any of the four different nitrogen sources which are NH_4SO_4, NH_4Cl, NH_4NO_3 and $NaNO_3$. Large scale (250 g) of sago waste SSF showed lower performance in terms of fiber and ash content and similar result on crude protein with small scale SSF (10 g).

This study has successfully evaluated the growth performance of red hybrid tilapia fed with fermented sago waste with the level of inclusion of up to 15% of fermented sago waste in the fish feed diet. The growth of red hybrid tilapia shows no significant difference as compared to red hybrid tilapia fed with the control diet. The fishes tested are also grown in good condition with no deleterious effect (fatal effect) shown up until the end of feeding trial experiments.

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