



# Feasibility of molasses-fermented banana peel as a protein source in practical diet for hybrid tilapia (*Oreochromis* spp.): effect on growth and feed efficiency

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**ABSTRACT.** This study aims to increase the utilization of banana peels, a local agricultural by-product, by improving nutrient value and partially reducing the presence of anti-nutritional factors (ANFs) as an alternative ingredient in fish feed. The feasibility of molasses-fermented banana peels (MFBP) as a protein source in a practical diet for hybrid tilapia (*Oreochromis* spp.) fries was investigated. Five isonitrogenous experimental diets containing 0 (control), 25, 50, 75, and 100% substitution levels of fish meal by MFBP were formulated. Twenty tilapia fries per group with three replicates were fed for eight weeks. The growth performance and feed efficiency values of fish were recorded and evaluated. Fish fed with 0, 25, and 50% MFBP levels showed significantly higher growth parameters, survival rate, and feed utilization efficiency than those fed with 75 and 100% MFBP levels. Although the control diet produced better growth performance and feed utilization efficiency than MFBP-based diets there was no significant difference between fish fed with the control diet and the low MFBP levels (0-50%) diet. Based on the present findings, it could be concluded that the molasses-fermented banana peels (MFBP) could efficiently replace the fishmeal by up to 50% without affecting the growth performance of red tilapia.

**Keywords:** fishmeal replacement; molasses-fermented banana peel; red tilapia; alternative feedstuff.

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## Introduction

In recent years, the aquaculture sector has been recognized as the fastest-growing food-producing industry and it is becoming increasingly significant in meeting the global demand for aquatic animal consumption (Food and Agriculture Organization [FAO], 2021). Thus, the aquaculture feed business has played an increasingly important role and grown in parallel. Therefore, fishmeal has been heavily used as the main protein source in the aquafeed industry because of its high protein content and balanced fatty acid profile (El-Sayed, 2020). Due to the high price and limitation of fish meal, which is produced from fish caught in the wild, it was substituted with various plant or grain by-products for the development of low-cost fish feed (Minjarez-Osorio et al., 2016; Daniel, 2018).

Ripe banana (*Musa sapientum* Linn.) peels are one of the agro-industrial-byproducts left behind from processing into various banana products by cooking, baking, or drying into dried bananas. Locally, the peels have been used as organic fertilizer, or simply discarded. Disposal of these peels might cause environmental problems (Pereira & Maraschin, 2015). Additionally, due to the abundant of phytochemicals, mainly polyphenols, carotenoids, flavonols, antioxidants, protein, carbohydrates, vitamins, minerals, and other bioactive compounds of banana peels, hence, they were used in animal feeding (Karaket, Somtua, Ponza, & Areechon, 2021; Kraithong & Issara, 2021; Naksing et al., 2021; Zaini et al., 2022; Likittrakulwong, Chanburee, Kitpot, Ninjaranai, & Pongpamorn, 2023). Although, in general, raw banana peels are utilized as an unconventional feed for livestock (Pimentel et al., 2016; Salim et al., 2021), anti-nutritional factors are one of the major problems associated with the use of raw plant by-products in fish feed (Baruah, Sahu, Pal, & Debnath, 2004).

From the past to the present, fermentation has typically been recognized as a safe and acceptable applied biotechnology, having been used in food processing and preservation. Generally, the culture of microorganisms on non-soluble materials or solid substrates commonly serves as both a nutritional supply

and physical support in a low-water-activity environment and is recognized as solid-state fermentation (SSF) (Sadh, Duhan, & Duhan, 2018). Fermentation is an inexpensive process for the degradation of anti-nutritional factors and thus improves the bioavailability of minerals, protein quality, and fiber digestibility of raw plant by-products (Mandal & Ghosh, 2020; Oguntoyinbo et al., 2020; Pratiwi & Pratiwy, 2021). Previous studies showed that the optimal dose of fermented plant by-products could be utilized in fish feed without affecting growth performance and/or other benefits. For instance, fermented water hyacinth was displayed to be a potential feed ingredient for river carp (*Leptobarbus hoevenii*) fingerling (Rahmad, Suharman, & Adelina, 2017), and common carp (*Cyprinus carpio*) (Sadique, Pandey, Khairnar, & Bt, 2018). Adewumi (2018) reported that the fermented cassava peel meal could thereby replace maize in *Clarias gariepinus* diets. Feed supplementation with fermented lemon peel has been shown to enhance growth performance, lysozyme activity, and immune response of orange-spotted grouper (*Epinephelus coioides*) (Zhuo, Chen, & Lin, 2021). Aisyah, Gustiningrum, Agustono, and Al-Arif (2021) demonstrated that fermented banana peel flour could be used as a substitute for commercial feed in Siam catfish (*Pangasius hypophthalmus*) culture. Furthermore, banana peels have enhanced the level of the macro- and micro-nutrients, mainly crude protein after fermentation, and exhibited the potential as animal feed ingredients (Pereira & Maraschin, 2015; Olorunnisola, Jamal, & Alam, 2018).

Therefore, the effects of molasses-fermented banana peels as a protein source in a practical diet on growth performance and feed efficiency of sex-reversed hybrid tilapia (*Oreochromis* spp.) fry, one of the most economically important farmed freshwater fish species worldwide, were investigated. This work would be beneficial for the potential application of banana peel which could contribute to the significant reduction of agricultural waste and by-products, and be useful for fundamental product development as an alternative beneficial plant-based protein source for the aquafeed industry in the future.

## Material and methods

### Fish and molasses-fermented banana peel preparation

Healthy all-male hybrid tilapia or red tilapia (*Oreochromis* spp.) fries with 2-3 cm in length were obtained from Phitsanulok Inland Fisheries Research and Development Center, Phitsanulok Province, Thailand. Fish were acclimatized in 500 L fiberglass tanks for 2 weeks before experimental manipulation and were fed with commercial powdered feed three times per day. Ripped banana peels, *Musa sapientum* Linn., were obtained from local dried banana factories at Amphoe Bang Krathum, Phitsanulok Province, Thailand. Then, it was washed and chopped into little pieces (2-3 cm) with a knife, then stored at 4°C in the refrigerator before being mixed with other ingredients to make MFBP. The media used for preparing the molasses-fermented banana peel (MFBP) consisted of 1 kg of the chopped banana peels, molasses 2.5, urea 3%, and 1 L of distilled water. The ingredients were incubated in the closed tank and mixed every week for 28 days. MFBP was dried in an oven at 60°C for 72 hour and then homogenized using a blender.

### Experimental design and diet preparation

A completely randomized design (CRD) was conducted to test the hypothesis of this experiment. The basal practical diet formula was received from the Nile tilapia philosophers in Thailand (Karaket, Seel-audom, & Areechon, 2021) for this testing. Five isonitrogenous experimental diets were formulated to contain 0 (control), 25, 50, 75, and 100% replacement of fishmeal with MFBP (referred to as MFBP0, MFBP25, MFBP50, MFBP75, and MFBP100, respectively) (Table 1 and 2). Other ingredients were optimized to make each treatment similar in nutritional value. The ingredients were mixed in a blender, the feeds were pressed through a 2 mm sieve in a pelleting machine, and the pellets were air-dried for 24 hours and stored in a refrigerator until use. The experimental diets were prepared biweekly and then stored in a refrigerator at 4°C for daily use. Proximate composition analysis (crude protein, ash, moisture, lipid, and fiber) and total tannin content for banana peel and test diets were performed using Association of Official Analytical Chemists (AOAC, 2000) methods.

### Experimental setup and data collection

The experiment was conducted at the Department of Agricultural Science, Naresuan University, Phitsanulok Province, Thailand. After acclimatizing of fish for two weeks, 30 fish were randomly distributed

into each group (200 L aquaria) with three replicates in the semi-closed greenhouse. Water quality was maintained as 4-6 mg L<sup>-1</sup> DO, pH 7-8, total ammonia below 0.5 mg L<sup>-1</sup>, and nitrite below 0.1 mg L<sup>-1</sup>. The aquaria were supplied with 50% water exchange with de-chlorinated freshwater supply weekly. The fish were fed with the experimental diets at the rate of 10% of body weight four times per day for 8 weeks. Five fish from each aquarium were randomly weighed for growth determination and feeding adjustment biweekly. The total feed consumed by fish was recorded.

After the 8<sup>th</sup> week of the feed trial, apparent digestibility coefficients (ADC) were carried out by the indirect method using chromic oxide (Cr<sub>2</sub>O<sub>3</sub>) as the non-absorbed reference substance.

In brief, the experimental diets as in Table 1 including 0.5% chromic oxide in each formula were fed to the fish for two weeks. Then, feces from each tank were collected once daily after the final meal of the day by siphoning. Immediately after the collection, feces from each tank were centrifuged, pooled, and stored at -20°C until analysis. Then, dried feces and feed were digested and determined for chromic oxide and crude protein (CP) by the colorimetric method as described in Lupatsch, Kissil, Sklan, and Pfeffer (1997).

At the end of the experiment, all survival fish from each replicate were counted and weighed in bulk. Growth performance, survival rate, and feed utilization were calculated by the following equations:

Weight gain (g) = final body weight – initial body weight;

Average daily gain (ADG, g fish<sup>-1</sup> day<sup>-1</sup>) = weight gain (g)/ experimental time (days);

Specific growth rate (SGR, % BW day<sup>-1</sup>) = 100 x (Ln final body weight - Ln initial body weight)/ experimental time (days);

Feed conversion ratio (FCR) = total feed intake/ weight gain;

Feed conversion efficiency (FCE, %) = 100 x (final body weight – initial body weight)/ dry feed consumed (g);

Protein efficiency ratio (PER) = wet weight gain (g)/ protein intake (g);

ADC of dry matter (%) = 100 – [(100 × Cr<sub>2</sub>O<sub>3</sub> of diet/ Cr<sub>2</sub>O<sub>3</sub> of feces)];

Survival rate (%) = 100 × (final number of fish/ initial number of fish).

**Table 1.** The proximate composition of banana peels used in formulating the experimental diets.

Parameter (% DM)	Banana peels	
	Unfermented	Fermented
Crude protein	9.62	32.10
Crude lipid	9.38	6.03
Fiber	15.14	6.13
Ash	14.58	12.85

**Table 2.** Feed formulation and nutrient composition of the experimental diets.

Items	MFBP0	MFBP25	MFBP50	MFBP75	MFBP100
	Feed formula (%)				
Fish meal	35.00	26.25	17.50	9.00	-
Soybean meal	35.00	35.00	35.00	36.00	37.50
Rice bran	10.00	10.00	9.00	5.00	1.00
Broken rice	10.00	10.00	9.00	5.00	1.00
Molasses-Fermented banana peels	-	13.80	27.50	43.00	58.50
Methylcellulose	8.00	2.95	-	-	-
Fish oil	1.00	1.00	1.00	1.00	1.00
Premix <sup>1</sup>	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00
Proximate composition (%)					
Dry matter	90.67	90.49	90.51	90.85	91.21
Crude protein	35.18	35.17	34.90	34.94	34.98
Crude lipid	9.38	8.70	7.73	6.08	4.82
Fiber	5.99	6.13	6.12	5.83	5.52
Ash	12.08	10.91	9.65	8.40	7.06
Nitrogen free extract <sup>2</sup>	28.04	29.58	32.11	35.60	38.83
Gross energy (MJ kg <sup>-1</sup> ) <sup>3</sup>	36.61	35.72	34.09	33.47	32.31

<sup>1</sup>Vitamin and mineral premix (kg<sup>-1</sup> diet): A, 5,000 IU; D3, 1,000 IU; E, 5,000 mg; K, 2,000; B1, 2,500 mg; B2, 1,000 mg; B6, 1,000 mg; B12, 10 mg; inositol, 1000 mg; pantothenic acid, 3,000 mg; niacin acid, 3,000 mg; C, 10,000 mg; folic acid, 300 mg; biotin, 10 mg; calcium phosphate, 80; calcium lactate, 100; ferrous sulfate, 1.24; potassium chloride, 0.23; potassium iodine, 0.23; copper sulfate, 1.2; manganese oxide, 1.2; cobalt carbonate, 0.2; zinc oxide, 1.6; magnesium chloride, 2.16; sodium selenite, 0.10. <sup>2</sup>NFE (nitrogen free extract) = 100 - (% moisture + % protein + % fat + % ash + % fiber). <sup>3</sup>Gross energy value was calculated based on combustion values of 23.7 protein, 39.5 fat, and 17.2 kJ g<sup>-1</sup> carbohydrates (Molina-Poveda, 2016).

### Statistical analysis

Fish growth performance and feed utilization efficiency were analyzed by one-way analysis of variance (ANOVA) and the means were compared using Duncan's New Multiple Range Test (DMRT). All data in this study were analyzed at a 95% confidence level by using the R program (R Core Team, 2019).

### Ethical statement

All animal care and handling procedures in the present study (project number NU-AQ650602) were approved by the Naresuan University Animal Care and Use Committee (certificate number 65 01 004).

## Results and discussion

Table 1 exhibited the proximate composition of the molasses-fermented banana peel (MFBP) used in formulating the experimental diets compared with the unfermented banana peel. The nutrient value, crude protein (32.1%) of the MFBP meal was higher than unfermented banana peel (9.62% CP). In contrast, crude lipid (6.03%), fiber (6.13%), ash (12.85%), and carbohydrate (42.89%) of the MFBP meal were lower than those of the unfermented banana peel (crude lipid, 9.38%; fiber, 15.14; ash, 14.58%; carbohydrate, 51.28%). This result also clearly demonstrated protein enrichment, and polysaccharides degradation in banana peels after solid-state fermentation (Olorunnisola et al., 2018; Oguntoyinbo, Olumurewa, & Omoba, 2020; Ozabor, Ojokoh, Wahab, & Aramide, 2020).

The results of growth, feed utilization, and survival rate of red tilapia throughout the experimental period of 60 days were listed in Table 3. The fish fed different diets were separated clearly at the sixth and eighth weeks with diet MFBP0 having a better growth pattern than any of the diets, this trend was followed by diets MFBP25, MFBP50, MFBP75, and MFBP100 being the lowest. The survival rate during the feeding trial was not significantly ( $p > 0.05$ ) affected by MFBP replacement in the diet. After the 60 day feeding trial, the effect of the MFBP-replacement diet was observed, where a further increase in MFBP levels caused a reduction in growth performance and feed efficiency. The highest final weight, weight gain, ADG, and SGR have been observed in fish fed the control diet (MFBP-free). However, there was no significant difference between fish fed with the control diet and the low MFBP levels (0-50%) diet ( $p > 0.05$ ). Moreover, fish fed with low MFBP levels and a control diet showed significantly higher final weight, weight gain, ADG, and SGR than those fed high MFBP levels (75-100%) diet ( $p < 0.05$ ). A similar tendency was observed for feed efficiency. The highest values were achieved by fish fed the control diet. Although the control diet produced better FCE, FCR, PER, and ADC of dry matter than MFBP-based diets there was no significant difference between fish fed with the control diet and the low MFBP levels (0-50%) diet ( $p > 0.05$ ). In addition, the feed efficiency of fish fed with a high MFBP (75-100%) levels diet was significantly lower than other groups ( $p < 0.05$ ).

**Table 3.** Growth performance, feed utilization, and survival rate of red tilapia fed with experimental diets for 60 days (mean  $\pm$  SD).

Parameters	Experimental diets				
	MFBP0	MFBP25	MFBP50	MFBP75	MFBP100
Initial weight (g)	0.52 $\pm$ 0.07	0.55 $\pm$ 0.14	0.55 $\pm$ 0.06	0.50 $\pm$ 0.12	0.48 $\pm$ 0.10
Final weight (g)	9.13 $\pm$ 0.35 <sup>a</sup>	8.53 $\pm$ 0.73 <sup>ab</sup>	8.52 $\pm$ 0.34 <sup>ab</sup>	7.69 $\pm$ 0.16 <sup>bc</sup>	6.53 $\pm$ 1.26 <sup>c</sup>
Weight gain (g)	8.61 $\pm$ 0.41 <sup>a</sup>	7.97 $\pm$ 0.36 <sup>ab</sup>	7.98 $\pm$ 0.67 <sup>ab</sup>	7.19 $\pm$ 0.16 <sup>bc</sup>	6.04 $\pm$ 1.16 <sup>bc</sup>
ADG (g day <sup>-1</sup> )	0.15 $\pm$ 0.01 <sup>a</sup>	0.14 $\pm$ 0.01 <sup>a</sup>	0.14 $\pm$ 0.01 <sup>a</sup>	0.13 $\pm$ 0.00 <sup>a</sup>	0.11 $\pm$ 0.02 <sup>b</sup>
SGR (% day <sup>-1</sup> )	5.13 $\pm$ 0.29	4.93 $\pm$ 0.43	4.89 $\pm$ 0.05	4.93 $\pm$ 0.38	4.62 $\pm$ 0.10
FCE (%)	44.93 $\pm$ 1.01 <sup>a</sup>	43.32 $\pm$ 1.41 <sup>ab</sup>	40.77 $\pm$ 0.67 <sup>b</sup>	36.46 $\pm$ 0.54 <sup>c</sup>	34.45 $\pm$ 2.80 <sup>c</sup>
FCR	2.23 $\pm$ 0.05 <sup>a</sup>	2.31 $\pm$ 0.08 <sup>a</sup>	2.45 $\pm$ 0.04 <sup>a</sup>	2.74 $\pm$ 0.04 <sup>b</sup>	2.92 $\pm$ 0.25 <sup>b</sup>
PER	1.21 $\pm$ 0.03 <sup>a</sup>	1.16 $\pm$ 0.04 <sup>a</sup>	1.08 $\pm$ 0.01 <sup>ab</sup>	0.97 $\pm$ 0.01 <sup>bc</sup>	0.84 $\pm$ 0.18 <sup>c</sup>
ADC of dry matter (%)	75.69 $\pm$ 2.01 <sup>a</sup>	70.66 $\pm$ 0.53 <sup>b</sup>	65.30 $\pm$ 1.46 <sup>c</sup>	60.87 $\pm$ 0.42 <sup>d</sup>	51.58 $\pm$ 1.66 <sup>e</sup>
Survival rate (%)	96.67 $\pm$ 3.33	96.67 $\pm$ 3.33	93.33 $\pm$ 8.82	90.00 $\pm$ 6.67	93.33 $\pm$ 3.33

Means in the same row followed by the same letter did not differ statistically according to DMRT's test ( $p > 0.05$ ).

Recent studies have shown the potential of using MFBP meal as an alternative ingredient to fish meal in red tilapia. These results are in agreement with a feeding trial that was conducted to evaluate the potential of using the whole banana meal as an alternative ingredient in the tambaqui (*Colossoma macropomum*), an omnivorous fish species. (Silva et al., 2020). On the other hand, inclusion rates of more than 30% of plant feedstuff in fish diets have typically resulted in reduced growth and poor feed efficiency (Wadhwa, Bakshi, &

Makkar, 2015; Daniel, 2018). In this study, up to 50% of MFBP meal was included in the diet of red tilapia without resulting in adverse effects on fish growth and feed utilization. Due to the degradation of anti-nutritional factors and fiber digestibility of banana peel using the fermentation process (Mandal & Ghosh, 2020; Oguntoyinbo et al., 2020; Pratiwi & Pratiwy, 2021), fish could utilize those diets. However, the inclusion of MFBP in diets at a rate of more than 50% showed lower energy (Table 2), which affected growth and feed efficiency. The results of apparent nutrient digestibility indicated the highest PER and ADC in fish fed with the control diet followed by the groups fed with low MFBP levels (0-50%) diet, and the group fed with high MFBP levels (75-100%) diet, respectively. These results are comparable to the results of a digestibility study conducted by Yossa, Fatan, Kumari, and Schrama (2022) reported that the apparent digestibility coefficient of protein and energy was the highest in the traditional diet followed by a plant-based meal diet in tilapia. The reason could be supplementing a low-energy diet would cause amino acid catabolism, which would supply enough energy for the normal metabolism of the animal, leading to low protein efficiency and slower growth (Molina-Poveda, 2016).

The tannic acid content of the banana peel and test diets was shown in Table 4. The reduction in tannin of MFBP may be due to the processing that the banana peel was subjected to coupled with the activities of microbial enzymes involved in the fermentation process (Ozabor et al., 2020). Thus, by increasing MFBP meal in the diets, the amounts of tannins were increased as shown. In the present study, the tannin content in the experimental diets ranged between  $0.04 \pm 0.01$  and  $0.18 \pm 0.01\%$ . The growth and feed utilization of fish were unaffected by tannic acid in all experimental groups. The tannin contents of this study did not exceed the 0.63% level to cause an impact on the growth and digestibility of dry matter, protein, and lipid (Pinto, Pezzato, Miranda, Barros, & Furuya, 2008).

**Table 4.** Tannic acid of banana peels and experimental diets (% , mean  $\pm$  SD).

Un-fermented banana peels	Molasses- fermented banana	Experimental diets				
		MFBP0	MFBP25	MFBP50	MFBP75	MFBP100
1.09 $\pm$ 0.01	0.34 $\pm$ 0.01	0 <sup>a</sup>	0.04 $\pm$ 0.01 <sup>b</sup>	0.09 $\pm$ 0.00 <sup>c</sup>	0.14 $\pm$ 0.01 <sup>d</sup>	0.18 $\pm$ 0.01 <sup>e</sup>

Different latter in experimental diets denoted statistically significant differences ( $p < 0.05$ ).

Typically, the anti-nutritional factors (ANFs) contained in raw materials should be considered when using alternative plant materials in animal feed. This is a limiting factor for their utilization in aquafeeds (Wadhwa et al., 2015). However, fermentation greatly decreased the anti-nutritional contents of the fermented banana peels, making the feed safe for fish (Ozabor et al., 2020), as well as in this present investigation. These demonstrated that some fermented plant sources could be used to completely replace soybean meal such as corn distiller dried grain in gilthead seabream, *Sparus aurata* (Diógenes et al., 2019), fermented soybean meal in Florida pompano, *Trachinotus carolinus* (Novriadi, Rhodes, Powell, Hanson, & Davis, 2018), and fermented jatropha kernel meal in rohu, *Labeo rohita* (Phulia et al., 2017). Consequently, solid-state fermentation (SSF) might be considered a better option for improving the nutritional value and decreasing the anti-nutritional factors of the alternative plant feedstuffs (Mandal & Ghosh, 2020).

## Conclusion

The present study demonstrated that the culture of red tilapia fry could be successfully carried out by using molasses-fermented banana peels (MFBP) as an alternative protein source in the diet. MFBP could efficiently replace the fishmeal up to 50% without affecting the growth performance and survival of this species under practical conditions.

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