



Effects of Feeds Containing Fermented Rice Bran and Sunflower Meal as Main Ingredients on Initial Growth and Body Composition of Tilapia Juveniles

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ABSTRACT

Rice bran, a by-product of the rice milling process, is often discarded as a wasted resource despite its high nutritional value. This study focused on the fermentation process and mixing of rice bran with other agricultural wastes to make effective use of rice bran as fish feed, and aimed to clarify the effects of feeds prepared by adding sunflower meal and soybean meal to rice bran and fermented rice bran on growth and proximate composition. In the experiment, Japanese rice bran was fermented with *Bacillus subtilis natto* and lactic acid bacteria to produce Unfermented Rice bran Feed (URF) and Fermented Rice bran Feed (FRF), which were compared with Commercial Feed (COF). Fermentation increased the crude protein content of rice bran and decreased the crude fat content; the results of a 28-day rearing trial showed that both URF and FRF had lower weight gain and SGR and worse feed efficiency than COF. On the other hand, the proximate composition of tilapia fed fermented rice bran was similar to that of commercial compound feed, suggesting that fermented rice bran is a more suitable feed for tilapia than unfermented rice bran. These results suggest that fermented rice bran is a promising feed for tilapia. Future research should investigate the growth-promoting effects of optimising the fermentation conditions and combining it with other feed ingredients.

Keywords: Fermented rice bran; Nile tilapia; Sustainable aquaculture; Feed formulation; Growth response

INTRODUCTION

Rice (*Oryza sativa* L.) is a highly nutritious crop, rich in carbohydrates, vitamins, and minerals, and can be stored for extended periods through drying [1]. It is widely cultivated, particularly in Asia, and serves as a staple food for approximately half of the world's population [2]. In recent years, the demand for rice in Europe and West Asia has increased due to the global popularization of Japanese cuisine. Rice cultivation as a cash crop is also expanding in the African region. The primary type of rice grown in the African region is upland rice, with African rice (*Oryza glaberrima* Steud.) and NERICA (New Rice for Africa), varieties adapted to low rainfall environments, being introduced as dominant cultivars [3].

When rice is processed for food, milling is commonly employed to remove the bran layer from unhusked brown rice [4]. The bran layer obtained from rice milling is rich in oils and fats, making it a potentially valuable raw material for oil extraction and other applications. However, adequate infrastructure for the collection,

transportation, and establishment of oil-pressing facilities is essential for utilizing this resource [5]. Consequently, in developing countries, particularly in areas with underdeveloped infrastructure, rice bran is often discarded. Therefore, the development of effective utilization technologies tailored to local conditions is urgently needed [6].

The effective use of rice bran as a raw material for fish feed has been reported [7]. Rice bran is rich in vitamins, minerals, and other nutrients beneficial to fish and is considered a promising protein source [8]. However, rice bran contains high levels of dietary fiber, which is difficult for fish to digest, and antinutritional factors such as phytic acid, which limits the inclusion of rice bran in feed to 10%-20% [9]. To address these limitations, treatments such as defatting and fermentation have been explored [10,11].

Fermentation is a process in which organic compounds are modified by microorganisms such as lactic acid bacteria, yeasts, and molds. Fermenting rice bran can improve nutritional components, including protein modification and crude fiber reduction [12].

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Furthermore, rice bran alone does not provide sufficient nutritional value as a complete feed and must be combined with other raw materials [13]. In this context, utilizing locally available agricultural waste, similar to rice bran, is desirable. Wheat and soybean meal, like rice bran, are organic wastes commonly used in fish feed. In Africa, sunflower oil production is prevalent, resulting in substantial quantities of sunflower meal, a byproduct of oil extraction, which could be utilized as feed [14].

This study aims to determine the effects of feeds composed of rice bran and fermented rice bran, in combination with sunflower meal and soybean meal, on the growth and body composition of juvenile Nile tilapia (*Oreochromis niloticus*), a species widely farmed in Africa and Asia.

MATERIALS AND METHODS

Fermentation of rice bran and production of feed

NERICA 10 rice bran cultivated in Japan was used as a feed ingredient. To ferment the rice bran, 20 kg of rice bran was mixed with a solution of 200 g of crushed *natto* and 400 g of commercially available yoghurt suspended in 500 ml of distilled water and, the top of the container was covered with a plastic sheet and kept in a constant temperature room at 30°C for 14 days. During the fermentation period, the rice bran was stirred every three days to maintain the internal temperature of the rice bran above 50°C, and distilled water was added as needed.

The end of fermentation was determined when the temperature of the rice bran fell below 30°C. Consequently, the fermentation was conducted at 30°C for 7-10 days under aerobic conditions. For the test feeds, unfermented or fermented rice bran was mixed with sunflower oil meal and soybean oil meal at levels of 50%, 35% and 15%, respectively, to produce Unfermented Rice bran Feed (URF) or Fermented Rice bran Feed (FRF). Commercial Feed (COF) was used as a control. The ingredients and nutrient composition of each diet are listed in Table 1. Note that moisture was analysed by heat drying method, crude protein by Kjeldahl method, crude fat by diethyl ether extraction method, crude fibre by filtration method, crude ash by direct ashing method and soluble Nitrogen-Free Matter (NFM) was calculated as residual.

Estimation of feeding rate

To estimate the feeding rate of the experimental feeds to tilapia, an assessment of the satiation rate was carried out; FRF was used in the study as there was no significant difference in the nutritional value of FRF and URF as feeds. Tilapia with average body weights ranging from 12 g to 100 g were divided into five test groups according to body weight and rearing trials were conducted in 30 L tanks (45 × 29 × 30 cm). The average weight and number of tilapias in each tank were 11.7 ± 2.8 g - 33 fish, 20.6 ± 2.8 g - 25 fish, 32.8 ± 5.2 g - 19 fish, 69.5 ± 7.5 g - 15 fish and 105.6 ± 8.4 g - 10 fish. The rearing water was tap water dechlorinated with activated carbon and supplied by a running water system. The water temperature was set at 28.0 ± 0.5°C, the optimum growth temperature for tilapia, and aerated at 600 mL/min to maintain a dissolved oxygen level of at least 4 mg/L. Fish were maintained for 7 days with a 12-hour light/dark cycle. Satiation was determined by feeding the maximum available amount of food three times a day for 30 min every three hours from the start of the light period and recording the total amount fed. The satiation rate was calculated from the amount of feed consumed and body weight [15].

Breeding trials with different feeds

In this study, juvenile tilapia with an average weight of 3.74.1 g were used as test fish and rearing experiments were conducted in 30 L tanks. The experimental period was 28 days and 15 fish were housed in each tank. Three test groups were established (COF, URF and FRF) and the experiment was conducted with two replications in each group. The rearing conditions were the same as in the satiation rate test described above, with water temperature maintained at 28 ± 1°C, dissolved oxygen concentration of 5.0 mg/L or higher and pH 7.0 ± 0.5. For feeding, the average body weight of the tilapia in each tank was measured once a week and the daily feeding rate was calculated based on the satiation rate. The calculated feeding rate was divided into three feedings at 10:00, 14:00 and 18:00 hrs. As COF, URF and FRF have different nutrient compositions, the feeding amounts were adjusted so that the crude protein feeding amounts were the same in each group. At the beginning and end of the rearing period, growth characteristics such as total length and body weight were measured and fish composition was analysed. At the end of the experiment, the test fish were frozen at -30°C and stored until analysis. Fish composition analysis was performed in the same way as for the diet.

RESULTS

Estimation of feeding rate

Throughout the rearing period, a stable rearing environment was maintained with a pH of 7.6 ± 0.07, a temperature of 27.6°C ± 0.10 and a dissolved oxygen concentration of 6.0 mg/L ± 0.28 in all tanks. The reared tilapia grew healthily without disease and no fish died. The fish weights and total feeds at the beginning and end of the experiment and the weight gain rate during the rearing period are shown in Table 2. Among the five test groups by body weight, the total feeding rate was approximately 200-210 g, except for the group with the highest initial body weight. The rate of weight gain tended to be higher in the groups with the lowest initial body weight, whereas in the group with the highest body weight, the total feeding rate was low and no increase in body weight was observed at the end of the experiment. The relationship between calculated fish weight and satiation rate is shown in Figure 1. The satiation rate was high in the group with the lowest initial body weight (4.47% ± 0.96%), but decreased slowly with increasing tilapia weight, reaching 1.62% ± 0.59% in the group with the highest initial body weight. The relational equation obtained from these results suggests that the feeding rate can be estimated by measuring the body weight of tilapia at regular intervals.

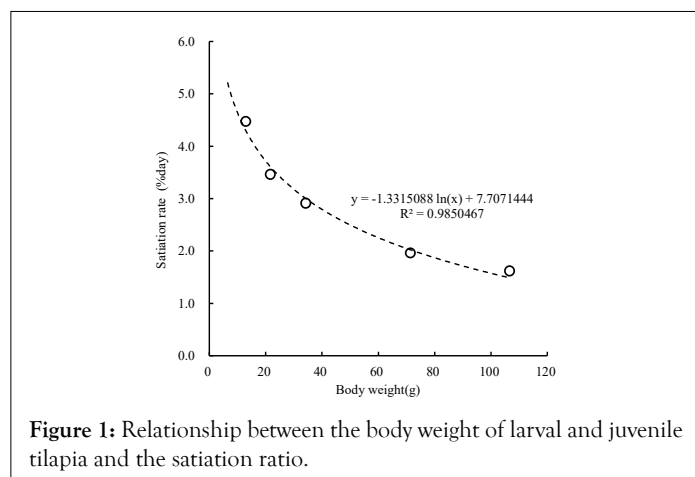


Figure 1: Relationship between the body weight of larval and juvenile tilapia and the satiation ratio.

Breeding trials with different feeds

In a 28-day rearing trial with three different feeds, the pH, water temperature and DO of each tank were maintained within the range of 7.4 ± 0.3 - 7.5 ± 0.2 , 24.9 ± 0.3 - 25.4 ± 0.5 and 8.1 ± 0.2 - 8.2 ± 0.1 , respectively, ensuring a stable growth environment. In all tanks, the tilapia grew healthily and the survival rate was 100%. Figure 2 shows the average body weight of each test group. During the first 14 days of rearing, the average body weights of the URF and FRF were similar to those of the COF, but from day 21 URF and FRF were significantly lower than the COF ($p < 0.05$). The average body weight on day 28 was significantly lower in the URF and FRF than in the COF, but no significant differences were found between the URF and FRF. There were no significant differences between URF and FRF. Table 3 shows the growth results at the end of the trial.

The highest increase in flesh weight was observed in the COF, while the URF and FRF were significantly lower than the COF

($p < 0.05$) no significant differences were observed between the URF and FRF, but the FRF showed a slightly lower trend; the COF showed the highest increase in flesh weight, while the URF and FRF showed a significantly higher increase in flesh weight than the COF ($p < 0.05$). Growth indices showed a similar trend, with the COF at about 2.8%/day compared to about 2.1%/day in the URF and about 1.7%/day in the FRF. Feed intake was about 1.8 times higher in the URF and FRF than in the COF, and FCR was significantly higher ($p < 0.05$) in the URF and FRF than in the COF. FCR was about 40% lower in the URF and 27% lower in the FRF than in the COF. Table 4 shows the composition of the fish at the end of the study. The crude protein content was highest in the COF at approximately 63%, followed by the FRF at approximately 55%, while the URF was low at approximately 50%. The crude fat content was approximately 20% in the COF and FRF, compared to approximately 34% in the URF, which was high. The ash content was highest in the COF, followed by the FRF and URF.

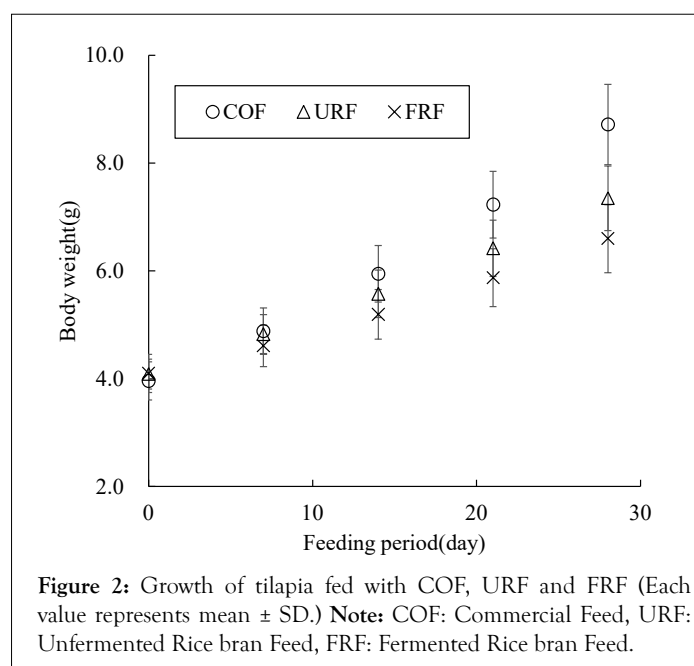


Table 1: Proximate composition of feed materials and testing feeds.

Item	Water Content (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	Crude Ash (%)	Nitrogen Free Extracts (%)
Rice bran	12.1	12.9	11.0	10.4	8.8	44.8
Fermented Rice bran	14.6	21.6	3.7	10.4	13.5	36.3
Sunflower meal	8.4	27.5	24.5	15.9	6.1	17.8
Soybean meal	11.8	45.0	1.9	6.4	5.3	29.5
COF	5.0	52.0	8.0	4.0	15.0	16.0
URF	10.8	26.0	13.4	7.9	10.4	31.5
FRF	14.3	27.5	9.7	9.8	9.5	29.3

Note: COF: Commercial Feed, URF: Unfermented Rice bran Feed, FRF: Fermented Rice bran Feed.

Table 2: Initial and final body weight of tilapia, total amount of feed, body weight gain, and survival rate during a 10-day feeding experiment under the satiation feeding.

Treatment number	Average body weight (g)		Total amount of feed (g)	Body weight gain (%)	Survival Rate (%)
	Initial	Final			
1	11.7 ± 2.8	14.4 ± 3.9	213.2	23.6	97
2	20.6 ± 2.8	23.0 ± 3.6	199.5	11.8	100
3	32.8 ± 5.2	36.1 ± 5.9	199.5	9.9	100
4	69.5 ± 7.5	73.8 ± 7.9	217.0	6.1	100
5	105.6 ± 8.4	107.9 ± 9.0	174.6	2.1	100

Table 3: Growth indices of tilapia fed with COF, URF and FRF.

Feed type	Feed intake (g)	Average body weight (g)		Weight gain (g)	SGR (%/day)	FCR	Feed efficiency	Survival Rate (%)
		Initial	Final					
COF	3.77 ± 0.35	3.96 ± 0.70	8.72 ± 1.49 ^a	4.76 ± 0.84 ^a	2.81 ± 0.17 ^a	0.82 ± 0.16 ^a	125.80 ± 22.18 ^a	100
URF	6.89 ± 0.12	4.09 ± 0.55	7.35 ± 1.20 ^b	3.26 ± 0.67 ^b	2.08 ± 0.18 ^b	2.21 ± 0.50 ^b	47.30 ± 9.65 ^b	100
FRF	6.60 ± 0.02	4.10 ± 0.71	6.60 ± 1.26 ^b	2.50 ± 0.62 ^b	1.69 ± 0.22 ^b	2.83 ± 0.81 ^b	37.93 ± 9.45 ^b	100

Note: SGR: Specific Growth Rate in weight, FCR: Feed Conversion Ratio Means in the same column having different superscripts are significantly different (p<0.05)

Table 4: Proximate nutritional composition of tilapia fed with COF, URF or FRF (mean ± SD).

Feed type	Water content (%)	Crude protein (%)	Crude fat (%)	Crude ash (%)
COF	75.61 ± 1.21 ^a	62.25 ± 2.11 ^a	17.08 ± 2.41 ^a	17.64 ± 0.55 ^a
URF	71.19 ± 1.25 ^b	50.28 ± 3.09 ^b	33.62 ± 2.75 ^b	11.13 ± 0.65 ^b
FRF	75.63 ± 1.13 ^a	55.05 ± 3.80 ^c	21.86 ± 1.63 ^a	16.99 ± 1.51 ^a

Note: COF: Commercial Feed; URF: Unfermented Rice bran Feed, FRF: Fermented Rice bran Feed.

Analysis was carried out on samples after lyophilisation.

Means in the same column having different superscripts are significantly different (p<0.05)

DISCUSSION

Rice bran has a long history of use as feed for livestock and farmed fish [4]. Commercially available aquaculture feeds are typically high in protein, with animal proteins such as fishmeal serving as the primary protein source [16]. Conversely, rice bran is an important feed resource in regions where low-cost aquaculture is prevalent. However, because rice bran contains antinutritional components like phytic acid, which can inhibit fish growth, and high concentrations of crude fat, which is easily oxidized, processing such as defatting and fermentation is essential for its effective use as feed

[9]. In omnivorous carp, the removal of phytic acid from defatted rice bran has been shown to improve growth and nutritional status, suggesting that fermentation treatment is also effective as an anti-nutrient measure [17].

In this study, fermentation treatment of rice bran with *Bacillus subtilis* var. *natto* and lactic acid bacteria resulted in an approximately 30% increase in crude protein content. This result was similar to the 32% increase in protein content observed in rice bran fermented with *Rhizopus oryzae* [18]. The increase in microbial biomass due to fermentation likely contributed to the rise in protein content.

Conversely, the crude fat content decreased by about 30% after fermentation. This contrasts with rice bran fermented with yeast, which exhibited a higher percentage of crude fat [17]. This difference may be attributed to *Bacillus subtilis natto*, a type of *Bacillus subtilis* with oil and fat decomposition capabilities, potentially degrading the oil and fat in the rice bran [19]. These findings suggest that fermentation treatment with *Bacillus subtilis natto* can suppress quality deterioration caused by oil and fat oxidation, a significant challenge in rice bran feed utilization. Compared to chemical degreasing, aerobic fermentation offers the advantage of lower costs, facilitating its implementation in areas with underdeveloped infrastructure.

In this study, tilapia larvae fed diets based on rice bran or fermented rice bran for 28 days showed significantly lower meat gain and Specific Growth Rate (SGR) compared to COF. The tested COFs likely promoted tilapia growth due to their high-quality animal protein sources, such as krill, squid, and fishmeal, as well as a balanced nutrient profile including vitamins and refined fish oil. The high Feed Conversion Ratio (FCR) observed in tilapia juveniles fed URF or FRF suggests that their elevated FCR may be related to their high protein content. Relying solely on these feeds for rearing remains a challenge [20]. The addition of 20% fermented rice bran to the diet of grey mullet (*Mugil liza*) larvae was reported to reduce growth, suggesting that higher levels of fermented rice bran in the diet may have a negative effect on growth in some fish species [21]. Studies involving FRF have shown improved growth in omnivorous carp species [22]. Tilapia growth has also been reported to improve when part of the commercial diet is replaced with fermented rice bran [23]. Furthermore, feeding fermented rice bran has been shown to increase yield in outdoor environments with diverse plankton populations [24]. Therefore, the appropriate feeding rate for fermented rice bran feed should be determined based on the fish species and aquaculture environment.

In this study, while no significant differences in tilapia growth were observed between the presence and absence of fermented rice bran, tilapia fed unfermented rice bran had lower crude protein and significantly higher crude fat content. This may be due to fat accumulation from the rice bran in the fish. Farmed fish are known to be highly susceptible to feed components, with oil and fat being particularly readily absorbed [25]. Conversely, tilapia fed fermented rice bran exhibited a proximate composition similar to commercial compound feeds, indicating that fermented rice bran is a more suitable feed for tilapia than unfermented rice bran.

Future studies are necessary to optimize fermentation conditions and to verify the growth-promoting effects of combining fermented rice bran with other feed ingredients. Evaluating the long-term effects of fermented rice bran feeding on growth and physiological functions is also crucial. Additionally, assessing the production costs and environmental impact of fermented rice bran is essential to determine its potential as a sustainable aquaculture feed resource.

CONCLUSION

This study evaluated the effects of feeds based on Japanese NERICA10 rice bran, sunflower meal, and soybean meal on the growth and body composition of juvenile Nile tilapia, an important aquaculture species in Africa and Asia. In particular, we focused on the fermentation treatment of rice bran and examined its effects by comparing it with unfermented rice bran.

The results showed that the Commercial off-the-shelf Feed (COF), with its high-quality animal protein sources and diverse nutrient composition, significantly promoted tilapia growth. In contrast, Unfermented Rice bran Feed (URF) and Fermented Rice bran Feed (FRF) resulted in lower growth rates compared to COF. However, it was confirmed that the fermentation treatment increased the crude protein content and decreased the crude fat content of rice bran. This suggests that fermentation by *Bacillus subtilis* has the potential to improve the nutritional composition of rice bran.

Tilapia fed URF showed an increase in crude fat content, suggesting fat accumulation in the fish body. This indicates that the fat content of rice bran is readily absorbed by the fish. In contrast, tilapia fed FRF showed a body composition statistically similar to those fed COF, suggesting that fermentation treatment may have optimized the nutritional balance of rice bran.

As demonstrated, this study suggests that fermentation treatment of rice bran has the potential to increase its value as a feed for tilapia. Future research should optimize fermentation conditions, and explore feed combinations, including long-term trials and cost-environmental impact assessments, to determine the feasibility and sustainability of this approach.

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