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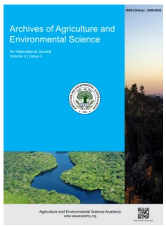

ORIGINAL RESEARCH ARTICLE

Effects of substituting plant-based protein sources for fish meal in the diet of Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

The purpose of this study was to evaluate the nutritional adequacy and suitability of rice polish and mustard oil cake as protein sources in the diet of Nile Tilapia (*Oreochromis niloticus*). To assess the growth performance and feed utilization of Nile Tilapia, three diets containing rice polish (0, 8, and 16%) and mustard oil cake (8, 16, and 24%) were formulated and fed to the fish over a period of 60 days. According to the findings, the growth performance tended to decline as the levels of rice polish and mustard oil cake increased. The control diet (30% Fish meal) resulted in the highest weight gain (373.79±49.78%), whereas the diet (20% Fish meal) resulted in the least weight gain (341.24±27.23%). The specific growth rate (SGR) followed the same pattern, and there were no statistically significant differences in SGR between diets (p>0.05). At the end of this trial, the feed intake (FI) of the various diets ranged between 32.37 g and 37.78 g per fish. Although feed conversion ratio (FCR) and protein efficiency ratio (PER) were not significantly different among diets (p>0.05), feed intake decreased as the incorporation of rice polish increased.

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INTRODUCTION

As the global population continues to increase, it is expected to surpass 8.0 billion by 2030. FAO predicts that seafood consumption will reach between 150 and 160 million tons annually by 2030. Due to the overexploitation of our fisheries resources, natural fish catches are declining; aquaculture will be required to fill this scarcity. A limited supply of fish meal from all available resources cannot meet the growing demand of the fish feed industry and producers (Tacon and Metian, 2009; Kaushik and Troell, 2010; Radhakrishnan *et al*., 2016). Therefore, plant-based protein appears to be the most suitable substitute for fish meal (FM) in tilapia diet formulation. Fish by-products, terrestrial animal by-products, and plant protein sources can serve as alternatives to FM. To satisfy the demand of a growing global population, it is anticipated that fish production will increase; this will necessitate the production of more fish feed. Due to its low cost and presence of balanced amino acids, the incorporation of plant-protein components in fish diets has increased (Gatlin *et al*., 2007; Naylor *et al*., 2009; Mahboob, 2014). With the continuous expansion of Tilapia production, the need for specialized diets incorporating locally produced ingredients has become a necessity. Feed is the single largest expense in semi-intensive and intensive fish cultivation, accounting for 30–70% of total operational costs (El-Sayed, 2004). The substitution of fishmeal with locally accessible and less expensive plant feedstuffs has been shown to be crucial for the future development of the aquaculture industry (Tacon *et al*., 2006). For fish culture in captivity, nothing is more important than the proper nutrition and nourishment. The Nile Tilapia is naturally adapted to consuming plant matter (Keenleyside, 1991). Ongoing research is being conducted to extensively determine the various protein sources as potential substitutes for fish meal in Tilapia diets. Cassava leaf meal (Ng and Wee, 1989), Rapeseed (Davies *et al.*, 1990), Barley and Lucerne (Belal, 1999), Legumes (Nyirenda *et al.*, 2000; Koumi *et al.*, 2009), and Ipil Ipil leaf (Zamal *et al.*, 2008) are examples of plant protein sources. These feed ingredients are palatable and nutritionally dense. Nonetheless, the rising cost of feed ingredients on the local market is the most significant issue facing the fish aquaculture industry. According to Zamal *et al.* (2009), it is essential that the ingredients used in the formulation of fish feed are both cost-effective and easily accessible within the local market.

Rice polish is a byproduct of the rice refining industry and is readily available year-round in Bangladesh, Vietnam, Thailand, China, and other rice-producing nations. During the milling process, the peri-cap, seed coat, nucleus, aleurone layer, germ, and a portion of the sub-aleurone layer of starchy endosperm are derived from the exterior layers of the rice caryopsis. Rice polish contains more protein than rice bran, red wheat flour, and maize meal, according to a number of studies (Saunders, 1990; Nyirenda *et al.*, 2000). According to previous research, rice polish contains 13-15% protein, 11-12% lipid, 40-45% nitrogen-free extract, as well as some essential minerals and vitamins (Saunders, 1990; Alencar and Alvarez, 1991). Nonetheless, this research was conducted to assess the possibility of incorporating rice polish and mustard oil cake as plant protein sources in Nile Tilapia diet formulation. The effects of these ingredients on the growth efficacy of Nile Tilapia are also investigated.

MATERIALS AND METHODS

The Nile Tilapia fry were collected from the local hatchery (Reliance Aqua Farms, Mymensingh) to conduct the experiment. The average initial weight was 4.52±0.24, 4.48±0.34 and 4.46±0.31 g in FM30, FM25 and FM20%, respectively. Two weeks were spent acclimating fish in a 40-liter plastic aquarium before feeding them 4-5% of their body weight with a diet containing 30% crude protein and 7.68% lipid. At the end of the acclimation period, each aquarium was stocked with 30 fish. Three replicate aquariums were used for each diet, and fish were fed 4-5% of their daily body weight three times in a day. The fish in each plastic aquarium were weighed in bulk at the beginning of the experiment and then weekly until the end of the feeding trial. A recirculation system that provided a continuous supply of aerated water at a rate of 1 L min-1 was employed for fish cultivation. To reduce disturbances, the rear and sides of every tank were covered with a 4 mm-thick layer of black plastic. According to Coyle *et al.* (2004), the provision of continuous illumination for a duration of 24 hours was maintained by the use of fluorescent ceiling lighting. During the experimental period, the water temperature, pH, and dissolved oxygen levels were measured in the experiment tanks. The temperature of the water was measured on a regular basis using a thermometer (Ree, 1953). The pH of water was measured using a digital pH meter. The amount of dissolved oxygen (DO) in water was determined using Winkler's method (Winkler, 1988). Temperature was 28.15±1.6˚C, dissolved oxygen was 5.6±0.3 mg L^{-1}, and the pH was 7.9±0.4. These conditions are appropriate for Tilapia cultivation (Stickney, 1986).

The proximate compositions of the experimental diets (protein, lipid, dietary fiber, ash, and hydration) were determined using the standard method (AOAC, 2000). To convert total nitrogen into crude protein, the Kjeldahl method with a conversion factor of 6.25 was used to analyze the crude protein. Using a Soxhlet extractor and petroleum ether, crude lipid was extracted (Siddique and Aktar, 2011; Siddique *et al.*, 2012). After assuring complete extraction, petroleum ether was evaporated and the residue was dried at 105°C to a constant weight. The fiber content was determined using 2.0g of samples that had been previously boiled with 0.3 N of diluted H_2SO_4. The mixture was filtered and then rinsed with 200 ml of boiling water and 0.5 N NaOH. The residue was re-extracted, washed with boiling distillate water and acetone, and then desiccated to constant weight at 105°C. The material was heated for three hours at 550°C, and its weight was recorded. The moisture content was determined by dehydrating feed samples to a constant weight at 105°C. The ash content was obtained through 4 hours of calcinations in a muffle furnace at 550° C. For the purpose of this study, the proximate chemical composition of a variety of feed ingredients that are commonly used in Bangladesh for the formulation of fish feed was analyzed, and the findings of the proximate chemical analysis are shown in Table 1.

The proximate chemical compositions of experimental diets are shown in Table 2. The proximate chemical compositions of the diets varied little. For optimal growth and nutrition efficiency, herbivorous Tilapia requires 30-35% of dietary protein (Ofojekwu *et al.*, 2003; Coyle *et al.*, 2004). Three experimental diets formulated for this investigation had the same crude protein content (30%). The formulation of the control diet, Diet FM-30, was developed using locally available ingredients and adhered to the standard formula commonly used in the local feed industry. Due to the partial substitution of rice polish and mustard oil cake, the amount of fish meal used in experimental diets was reduced by up to 10 %. The experimental diets FM-30 (control), FM-25, and FM-20 contained 30%, 25%, and 20% fish meal, respectively. The quantity of soybean meal, mustard oil cake and maize was calculated by a computer solver application. The market price of mustard oil cake is comparatively lower; however, its protein content is larger (26.79%) in comparison to maize and red wheat flour. To maintain the total protein level (30%) in the experimental diets, the proportion of mustard oil cake was increased with rice polish in FM-25 and FM-20, while rice polish and mustard oil cake were used to supplant maize in large quantities. Vitamin and mineral premix (0.2% of the diet) was added to all experimental diets as a vitamin and mineral supplement and balancer. For the preparation of the diets, the ingredients were coarsely ground and thoroughly mixed. The water was added to attain a moisture content of 25%. The diets were then passed through a mincer with a die, formed into

filaments with a diameter of 0.4 mm, and air-dried at 24°C for 24 hours. The dry rations were crushed and sieved into pellets of the proper size for fish (Luquet, 1991). The granules were stored at a temperature of -20°C until they were ready for use. The crude protein, lipid, fiber, ash, and hydration contents of duplicate samples of each diet were analyzed.
Under experimental conditions, ion-exchange chromatography analyzed amino acids from three meals. The 5.0 mg of protein was hydrolyzed with 1.0 mL of 6 N HCl in vacuum-sealed hydrolysis vials at 110°C for 22 hours. The HCl was internal standardized using norleucine. Hydrolysis vials were opened and stored in a desiccator with NaOH pellets under vacuum for 5-6 days to dry. The residue was dissolved in an appropriate volume of sample dilution Na-S buffer and filtered through 0.22 µm pore size membrane. A Beckman equipment (model 7300, USA) with an automatic integrator analyzed amino acids using ion-exchange chromatography. The recovery of protein nitrogen involved the conversion of ammonia resulting from the degradation of amino acids during acid hydrolysis (Mosse, 1990).

The following formulae calculated growth performance:

BWG (Body Weight Gain) = [(Final Weight - Initial Weight)/ initial weight] x 100

SGR (Specific Growth Rate, %/day) = (In Final Body Weight - In Initial Body Beight)/No. of Days

FI (Feed Intake) = Total Feed Intake Per fish (g) / Days.

FCR (Feed Conversion Ratio) = dry feed intake/weight growth.

(PER) Protein Efficiency Ratio = Weight Gain (g)/Protein Intake (g).

Statistical analysis

To determine the effect of different diets on the growth of Tilapia, a one-way analysis of variance (ANOVA) was performed, and Tukey's multiple comparison test was used to evaluate differences between means at $p<0.05$. SPSS Statistical Package (Version 24.0, SPSS Inc., Chicago, IL) was utilized to conduct all statistical analyses of the collected data. The statistical significance was examined with a confidence level of 95%.

Table 1. Proximate chemical composition of different feed ingredients used in Bangladesh.

Ingredients	Protein (%)	Lipid (%)	Fiber (%)	Ash (%)	Moisture (%)
Fish meal	51.48	4.76	3.43	22.76	9.21
Rice polish	17.31	2.96	7.14	9.63	9.49
Mustard oil cake	26.79	2.78	7.46	16.31	7.73
Maize	7.68	3.83	3.24	2.87	5.52
Red wheat flour	17.28	2.61	2.16	1.98	12.88
Soybean meal	43.59	4.38	5.65	5.78	11.75

Table 2. Formulation and proximate composition of the experimental diets.

	FM-30 (Control)	FM-25	FM-20
Ingredients used in the diet (%)			
Rice polish	0	8	16
Fish meal	30	25	20
Soybean meal	28	28	30
Mustard oil cake	8.0	16	24
Maize	24	14.2	3.8
Red wheat flour	9.8	8.6	6.0
Vitamin and mineral premix	0.2	0.2	0.2
Proximate composition (% of dry matter basis)			
Crude Protein	30	30	30
Lipid	7.68	8.37	9.15
Fibre	5.37	6.97	7.74
Moisture	8.39	8.98	9.38
Ash	14.98	18.32	18.96

FM= Fish meal

RESULTS AND DISCUSSION

Despite the fact that all three of the experimental meals had 30% protein, the FM-20 diet had the highest levels of crude fat and fiber. This was because it contained a higher quantity of soybean meal and mustard oil cake. To meet the essential amino acid (EAA) requirements of Tilapia, the essential amino acid (EAA) concentrations of all of the diets were adequate, with the exception of methionine (Table 3). The composition of essential amino acids in three distinct experimental diets, as well as the essential amino acid requirements of Tilapia, are shown in Table 3. At the end of the experiment, the growth performances of all Nile Tilapia that were fed experimental meals were measured and analyzed. Table 4 displays the growth response of Nile Tilapia fingerlings in terms of their initial and final mean body weights, percentage body weight gain (BWG), and specific growth rate (SGR). The survival rate was high in all treatment diets through the end of the trial period, and it ranged from 91.47±2.12 to 94.56±2.15% (Table 4). With the exception of the final weight of fish fed diet FM-20, which included larger inclusions of plant protein (rice polish and mustard oil cake), there were no significant differences ($p>0.05$) among the treatments. However, there was a general trend towards a decline in growth performance as the inclusion level of rice polish increased. Diet FM-20 had the lowest body weight gain (341.24±27.23%) compared to the control diet (FM-30), which had the highest BWG (373.79±49.78.%). The observed specific growth rate (SGR) exhibited a consistent trend, consistent with previous findings. At the end of the trial, the amount of food consumed by each fish varied between 32.37 and 37.78 gm depending on the type of diet. The amount of feed consumed was substantially higher ($p<0.05$) in the diets FM-30 and FM-25 compared to the diet FM-20. In spite of the fact that the feed conversion ratio (FCR) did not substantially differ ($p > 0.05$) among the diets, the amount of feed consumed by the animals decreased as the proportion of rice polish in their meals increased.

According to Zamal *et al.* (2008) and Koumi *et al.* (2009), the selection of feed ingredients is one of the most significant considerations in the formulation and commercial production of supplemental quality feed for any aquatic species. This is because feed ingredients are the building blocks of the feed. Even though fish meal is one of the most common feed ingredients used as an animal protein source and is widely recognized for having a greater protein content as well as important amino acids, it is significantly more expensive than the many plant-based sources of protein that are currently accessible (Vechklang *et al.*, 2011). In this study, we selected the ingredients after evaluating a variety of factors, including their nutritional value and relative cost. As a consequence of this, 8–16% rice polish and 16-24% mustard oil cake was incorporated into the diets in order to partially complement the fish meal. According to Ofojekwu *et al.* (2003), the optimal growth of fish fry requires a protein intake of between 35 and 55% of their total calories. Studies have shown that herbivores have lower requirements for the amount of protein in their diets compared to carnivores. In the present study, the SGR were not varied among the diets due to maintain same amount of crude protein (30%) in all experimental diets. On the other side, the high fiber content of FM-20, which was achieved by adding 20% rice polish and 22% mustard oil cake, could be responsible for the low feed intake of FM-20. According to the findings of a number of studies, a low food conversion ratio (FCR) indicates that produced feed is being utilized efficiently. According to Coyle *et al.* (2004) and Zamal *et al.* (2009), the diet with the highest amount of energy led to the lowest FCR and the highest amount of nutrient retention. Similarly, the protein efficiency ratio, indicates the proportion of protein retained after conversion. According to Koumi *et al.* (2009), FCR and PER are both related to the quantity of dietary protein consumed by fish and how this protein is converted into weight gain. In this study, there was no significant difference in terms of FCR or PER between the three experimental diets that were consumed by Tilapia fish. Therefore, partial replacement of fish meal with rice polish (up to 20%) and mustard oil cake (up to 22%) would be economically efficient because it would reduce the cost of feed formulation without affecting the nutritional content.

Table 3. Essential amino acid composition of different experimental diets and essential amino acid requirement of Tilapia species.

Essential amino acid composition (% of dietary protein)	EAA requirements for Tilapia (NRC, 1991)	FM-30	FM-25	FM-20
Arginine	4.20	5.36	5.68	6.48
Histidine	1.70	2.47	2.28	2.19
Isoleucine	3.10	3.66	3.76	3.87
Leucine	3.40	6.64	6.38	5.96
Lysine	5.10	6.43	6.85	6.17
Methionine	2.70	2.72	2.42	1.86
Phenylalanine + Tyrosine	3.80	6.36	6.59	5.98
Threonine	3.80	4.38	3.90	3.78
Valine	2.80	4.21	3.98	4.20

Table 4. Growth, survival and feed utilization of Nile Tilapia fed experimental diets for 60 days (mean ± SD).

Parameters	FM-30	FM-25	FM-20
IBW (g)	4.52±0.24[a]	4.48±0.34[a]	4.46±0.31[a]
FBW (g)	29.19±1.56[a]	23.16±0.69[a]	20.41±0.88[b]
BWG (%)	373.79±49.78[a]	367.19±61.43[a]	341.24±27.23[b]
SR (%)	94.56±2.15[a]	93.46±2.19[a]	91.47±2.12[a]
SGR (% day^{-1})	4.09±0.38[a]	4.06±0.42[a]	3.90±0.31[b]
FI	37.78±3.42[a]	36.03±3.81[a]	32.37±3.73[b]
FCR	2.31±0.54[a]	2.29±0.31[a]	2.28±0.35[a]
PER	1.39±0.19[a]	1.37±0.16[a]	1.35±0.28[a]

Values within the same row with different letters are significantly different (P<0.05).

Conclusion

As the levels of rice polish and mustard oil cake increased, the growth performance tended to decline. The control diet (30% Fish meal) revealed the highest weight gain (373.79±49.78%), while the diet (20% Fish meal) resulted the least weight gain (341.24±27.23%). There was no statistically significant differences in the specific growth rate (SGR) between diets (p>0.05). At the end of this experiment, the feed intake (FI) ranged between 32.37g and 37.78g per fish for the different diets. Although feed conversion ratio (FCR) and protein efficiency ratio (PER) did not differ significantly among diets (p>0.05), feed intake decreased as rice polish was increased. There was no statistically significant difference in FCR or PER between the three experimental diets fed by Tilapia fish in this study. Therefore, partial replacement of fish meal would be economically efficient because it would reduce the cost of feed formulation without compromising nutritional value.

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