Replacement of fish meal by plant protein sources in Nile tilapia (*Oreochromis niloticus*) diet: growth performance and utilization

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Abstract

The nutritional suitability and cost effectiveness of rice polish and mustard oil cake as protein sources in the diet of Nile tilapia (*Oreochromis niloticus*) were studied. This study introduced rice polish as a plant protein source for Nile tilapia and three diets were formulated using rice polish (0, 10 and 20%) and mustard oil cake (10.0, 17.6 and 22.0%) for a feeding trail of eight weeks to observe the growth performance and feed utilization. The result was indicated that growth performance tended to decrease with increase in inclusion level of rice polish and mustard oil cake. The control diet (FM35) recorded the highest body weight gain (BWG) (363.79±59.32%) and the least (330.24±32.32%) was in diet FM25. Specific growth rate (SGR) was followed the same trend and no significant differences of SGR was observed among the diets (P>0.05). Feed intake (FI) of different diets was ranged between 30.33 g and 35.08 g per fish at the end of this experiment. Feed intake was also declined with the increase in inclusion level of rice polish, though the feed conversion ratio (FCR) and protein efficiency ratio (PER) were not significantly different (P>0.05) among the diets. The results of this study revealed that partial replacement of fish meal by rice polish and mustard oil cake would be cost effective without any significant change in growth performance.

Keywords: Rice polish, mustard oil cake, plant protein sources, *O. niloticus*, growth performance

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Introduction

As the global population continues to grow and forecasts exceeding 8 billion by the year 2030. The seafood consumption is predicted to reach between 150-160 million tonnes per year in 2030 (FAO, 2005). The decline in wild fish catches due to overexploitation of our fisheries resources; aquaculture will be called upon to fill this gap. Aquaculture production is mostly destined for human consumption and 45.7% of the world's fish was accounted for human consumption in 2008 (FAO, 2010). Asian countries were accounted for 88.8% of world aquaculture production by quantity, where freshwater fishes were continued to dominate (54.7%) the total fish production by quantity (FAO, 2010).

Nile tilapia (*O. niloticus*) is one of the most important herbivorous fish species in world aquaculture. According to the Fishery Statistical Yearbook of Bangladesh (DoF, 2012), tilapia production in Bangladesh was about 66,767 tonnes in 2007. During 1999 to 2007, there was a tremendous progress in tilapia farming in Bangladesh. With the continued growth of tilapia production, the need for suitable diets tailored using local ingredients that are produced within each country has become a necessity.

Feed is the single largest expenditure in semi-intensive and intensive fish culture and feeding cost accounts about 30-70% of the total operational cost in a fish farm (El-Sayed, 2004). The replacement of fishmeal with locally available and cheaper plant feedstuffs is proved to be very essential for the future development of aquaculture sector (Tacon et al., 2006). For culturing fish in captivity, nothing is more important than the sound nutrition and adequate feeding. Nile tilapia is naturally accustomed to eating plant ingredients (Keenleyside, 1991). A substantial amount of research is already underway, testing potential protein sources that can replace fish meal in tilapia diets. These plant protein sources include cassava leaf meal (Ng and Wee, 1989), rapeseed (Davies et al., 1990), barley and alfalfa (Belal, 1999), soybeans (Nyirenda et al., 2000; Koumi et al., 2009), ipil ipil leaf (Zamal et al., 2008). These feed stuffs have good palatability and high nutritional quality. However, the major problems confronting the fish farming industry are the increasing cost of feed ingredients in the local market. Selection of ingredients for the formulation of fish feed is very crucial and it should be cheap and available in the local market (Zamal et al., 2009).

Rice polish is a by-product of rice milling industry and available throughout the year in Bangladesh, Vietnam, Thailand, China and other rice producing countries. It derived from the outer layers of the rice caryopsis during milling process and consists of peri-cap, seed coat, nucleus, aleurone layer, germ and part of sub-aleurone layer of starchy endosperm (Juliano, 1988). A number of studies were showed that rice polish contains higher amount of protein than the rice bran, red wheat flour and maize meal (Saunders, 1990; Nyirenda et al., 2000). Previous studies were revealed that rice polish contains 13-15% of protein, 11-12% of lipid, 40-45% of nitrogen free extract, some essential mineral and vitamins (Saunders, 1990; Alencar and Alvarenger, 1991). However, this study was conducted to evaluate the potentiality for utilizing the rice polish and mustard oil cake as plant protein source in diet formulation of Nile tilapia. The effects on growth performance of Nile tilapia by using these ingredients are also investigated.

Materials and methods

Nile tilapia was collected from a local hatchery (Rupali Fish and Prawn Hatchery Limited, Rawzan, Chittagong). The mean total length (±SD) and body weight (±SD) of experimental fishes were 5.32±0.050 cm and 4.24±0.106 g, respectively. Fishes were acclimated for two weeks in plastic tank (50 litres of volume) and fed at 3-4% of their body weight with control diet containing 35% crude protein and 6.98% lipid. At the end of acclimated period, fish were counted and stocked at density of 30 fish per tank. Three replicate tanks were used for each diet and fishes were fed three times (07:00, 12:00 and 17:00 h) at a rate of 3-4% of their body weight per day. In Bangladesh, farmers usually use fixed feeding for tilapia culture due to reduce the feeding cost. Therefore we did not use satiated feeding for our experiment. Fishes from each plastic tank were bulk weighed at the beginning of the experiment and again every week until end of the feeding trial. Fishes were reared in a continuous recirculation system that was supplied with aerated water at a flow rate of 1 L min^{-1}. Black plastic (4mm) was used to cover the back and sides of all tanks to minimize disturbances. Illumination was supplied with florescent ceiling lights with 24-hours light (Coyle et al., 2004). During the experimental period water temperature, pH and dissolved oxygen of the experiment tanks were measured regularly. Water temperature was recorded by using thermometer (Ree, 1953). Water pH was recorded by using a digital pH meter. The dissolved oxygen content (DO) of water was determined by Winkler's method (Winkler, 1988). Overall means for water quality parameters were: temperature, 27.5±1.2˚C; dissolved oxygen, 5.8±0.4 mg L^{-1} and pH 8.1±0.2. These values are within suitable conditions for tilapia culture (Stickney, 1986).

The proximate compositions (protein, lipid, dietary fibre, ash and moisture contents) of the experimental diets were determined according to the standard method (AOAC, 2000). The crude protein was analyzed by the Kjeldahl method with a conversion factor of 6.25 to convert total nitrogen into crude protein. Crude lipid was extracted by Soxhlet extractor with petroleum ether (Siddique and Aktar, 2011; Siddique et al., 2012). After ensuring complete extraction, petroleum ether was evaporated and the residue was dried to a constant weight at 105° C. Fibre was quantified on 2 g samples previously boiled with diluted H_2SO_4 (0.3 N). The mixture was filtered and washed with 200 ml of boiling water and NaOH (0.5 N). The residue was re-extracted, after washed with boiling distilled water and acetone and finally dried at 105˚C to constant weight. The material was heated at 550˚C for 3 h and the weight recorded. The moisture content was determined by drying the feed samples in an oven at 105˚C until a constant weight was obtained. The ash content was obtained by calcinations in a muffle furnace at 550˚C for 4 h. Proximate chemical composition of different feed ingredients used in Bangladesh for fish feed formulation were analyzed for this study and the result of proximate chemical analysis is presented in Table 1.

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

Ingredients	Protein (%)	Lipid (%)	Fibre (%)	Ash (%)	Moisture (%)
Rice polish	16.63	2.81	6.64	9.33	9.72
Fish meal	51.43	4.86	3.37	21.82	8.91
Soybean meal	42.56	4.62	4.26	5.45	11.85
Mustard oil cake	26.68	2.71	7.61	15.42	6.73
Maize	8.50	3.53	2.24	1.90	4.32
Red wheat flour	16.22	1.76	1.36	1.73	11.87

Proximate chemical compositions of experimental diets are presented in Table 2. The proximate chemical compositions were varied little among the diets. As an herbivore, tilapia requires 30-35% of dietary protein for their optimal growth and feeding efficiency (Ofojekwu et al., 2003; Coyle et al., 2004). Three experimental diets formulated for this study were iso-nitrogenous in terms of crude protein (35%). Diet FM35 (control) was formulated from practical ingredients according to a model formula used in the local feed industries. The quantity of fish meal used in the experimental diets was reduced up to 10% due to the partial replacement of rice polish and mustard oil cake. However, 35%, 30% and 25% of fish meal were used in the experimental diets FM35 (control), FM30 and FM25; respectively. The quantity of soybean meal, mustard oil cake and maize was considered by computer solver package programme. The market price of mustard oil cake is cheaper and contains higher percentage of protein (26.68%) than maize and red wheat flour. The percentage of mustard oil cake was increased with rice polish in FM30 and FM25, while maize was greatly replaced by rice polish and mustard oil cake to maintain the total protein level (35%) in the experimental diets. Vitamin and mineral premix (0.2%) were used in all experimental diet as a supplement of vitamin and minerals and to make it balanced. To prepare the diets, ingredients were ground into small particle sizes and mixed thoroughly. Water was added to obtain a 25% moisture level. Diets were then passed through a mincer with a die and made into 0.4 mm diameter strands and air dried (24° C) for 24 h. Dry diets were broken up and sieved to an appropriate pellet size for the fish (Luquet, 1991). The dry pellets were stored in frozen (-20 °C) condition until use. Duplicate samples of each diet were analyzed for crude protein, lipid, dietary fibre, ash and moisture contents.

Table 2: Formulation and proximate composition of the experimental diets

	FM35 (Control)	FM30	FM25
Ingredients used in the diet (%)			
Rice polish	0	10	20
Fish meal	35	30	25
Soybean meal	25	25	27
Mustard oil cake	10	17.6	22
Maize	21.2	9.2	0.8
Red wheat flour	8.6	8.0	5.0
Vitamin and mineral premix	0.2	0.2	0.2
Proximate composition (% of dry matter basis)			
Crude Protein	35	35	35
Lipid	6.98	7.67	8.59
Fibre	4.91	5.27	5.84
Moisture	7.19	7.58	7.92
Ash	13.93	16.64	16.82
Feed formulation cost [1]	46 BDT/kg	39 BDT/kg	35 BDT/kg

Note: [1]1 USD = 81.79 BDT (Accessed 17 September, 2012). http://www.xe.com/ucc/convert/?Amount=1&From=USD&To=BDT).

Amino acid analysis for three experimental diets was carried out by ion-exchange chromatography under the experimental conditions. For protein hydrolysis, samples containing 5.0 mg of protein were hydrolyzed with 1.0 mL of 6 N HCl in vacuum-sealed hydrolysis vials at 110°C for 22 h. Norleucine was added to HCl as an internal standard. After hydrolysis, the hydrolysis vials were opened and stored for five to six days in a desiccator containing NaOH pellets under vacuum until dry. Then the residue was dissolved in a suitable volume of a sample dilution of Na-S buffer and filtered through a Millipore membrane (0.22 µm pore size). The amino acids were analyzed by ion-exchange chromatography in a Beckman instrument (model 7300, USA) equipped with an automatic integrator. The ammonia content was included in the calculation of protein nitrogen retrieval, as it comes from the degradation of some amino acids during acid hydrolysis (Mosse, 1990).

Growth performance values were calculated by using following equations:

BWG (body weight gain) = [(final weight - initial weight)/initial weight] × 100

SGR (specific growth rate, %/day) = (ln final body weight - ln initial body weight)/no. of days × 100

FI (Feed intake) = Total feed intake per fish (g) /no. of days

FCR (food conversion ratio) = dry feed intake (g)/weight gain (g)

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

One way analysis of variance (ANOVA) was used to determine the effect of different diets on the growth of tilapia and Tukey's multiple comparison test applied to evaluate differences between means at $p < 0.05$. All the statistical analyses of the collected data were performed by using SPSS Statistical Package (Version 16.0, SPSS Inc., Chicago, IL). Statistical significance was tested at 95% confidence level.

Results

Although, the three experimental diets had 35% of protein, FM25 had the highest level of crude lipid and fibre due to using higher amount of soybean meal and mustard oil cake. Except for methionine, the essential amino acid (EAA) contents of all the diets were sufficient to satisfy the EAA requirements for tilapia (Table 3). Essential amino acid composition of three experimental diets and the EAA requirement of tilapia were presented in Table 3.

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, 1993)	FM35	FM30	FM25
Arginine	4.20	5.32	5.62	6.41
Histidine	1.70	2.37	2.18	1.91
Isoleucine	3.10	3.86	3.61	3.20
Leucine	3.40	6.65	6.18	5.87
Lysine	5.10	6.23	6.65	6.07
Methionine	2.70	2.72	2.45	1.99
Phenylalanine + Tyrosine	3.80	6.26	6.49	5.89
Threonine	3.80	4.23	3.94	3.84
Valine	2.80	4.11	3.94	4.03

The growth performances of all Nile tilapia that were fed experimental feeds were determined at the end of eighth week. Growth response of Nile tilapia fingerlings are presented as initial and final mean body weights, percentage body weight gain (BWG), and specific growth rate (SGR) in Table 4. Through the end of the experimental period, the survival rate was high in all treatment diets and ranged from 89.44±1.55 to 91.47±2.75% (Table 4). There were no significant differences (P>0.05) among treatments, with the exception of the final weight of fish fed diet FM25, which had higher inclusions of plant protein (rice polish and mustard oil cake). However, growth performance tended to decrease with increase in inclusion level of rice polish. The control diet (FM35) recorded the highest BWG (363.79±59.32%) and the least (330.24±32.32%) was diet FM25. SGR followed the same trend in this study. Feed intake of the different diets ranged between 30.33 g and 35.08 g per fish at the end of the experiment. Intake of feed was significantly higher ($p < 0.05$) in diet FM35 and FM30 than that of diet FM25. Feed intake was declined with the increase of rice polish, though the feed conversion ratio (FCR) was not significantly different ($p > 0.05$) among the diets (Table 4).

Table 4: Growth, survival and feed utilization of Nile tilapia fed experimental diets for 8 weeks (mean ± SD)

Parameters	FM35	FM30	FM25
IBW (g)	4.22±0.24	4.35±0.34	4.14±0.31
FBW (g)	19.53±1.79^{a}	19.79±0.39^{a}	17.75±0.65^{b}
BWG (%)	363.79±59.32	357.47±39.82	330.24±32.32
SR (%)	90.60±3.20	89.44±1.55	91.47±2.75
SGR (% day^{-1})	2.73±0.36	2.71±0.46	2.60±0.41
FI	35.03±3.46^{a}	35.08±3.91^{a}	30.33±3.93^{b}
FCR	2.31±0.52	2.27±0.29	2.23±0.33
PER	1.38±0.19	1.36±0.16	1.32±0.28

Note: Values are means ± SD. Values within the same row with different letters are significantly different (P<0.05).

Discussion

Selection of feed ingredients is one of the most important factors for the formulation and commercial production of supplemental quality feed for any aquatic species (Zamal et al., 2008; Koumi et al., 2009). Although, fish meal is the widely used feed ingredients as animal protein source and accepted for its higher protein composition and essential amino acids; but it is rather expensive than the available plant protein sources (Vechklang et al., 2011). In the present study, ingredients were selected to consider their nutritional quality and also their cost effectiveness. Therefore, 10-20% of rice polish and 17.6-22.0% mustard oil cake were used in the diets as a partial supplement of fish meal. Protein requirement of juvenile fishes is ranged from 35 to 55% for their optimal growth (Ofojekwu et al., 2003). A number of studies showed that herbivores require fewer amounts of proteins than the carnivores. In the present study, the SGR were not varied among the diets due to maintain same amount of crude protein (35%) in all experimental diets. On the other hand, the low feed intake of FM25 could be attributed to the high fibre content due to inclusion of 20% of rice polish and 22% of mustard oil cake.

A number of study argued that low food conversion ratio (FCR) value is an indicator of feed utilization efficiency of formulated feed. The high-energy diet produced the lowest FCR and the highest nutrient retention (Coyle et al., 2004; Zamal et al., 2009). Similarly, protein efficiency ratio (PER) is used as an indicator of protein retention of conversion ratio. FCR and PER both are related to dietary protein intake and its conversion into fish weight gain (Koumi et al., 2009). In the present study, there were no significant effect on FCR and PER among the three experimental diets fed by tilapia fish. Rice polish (8BDT/kg) and mustard oil cake (19 BDT/kg) are very cheap in the local markets of Bangladesh. Therefore, partial replacement of fish meal by rice polish (up to 20%) and mustard oil cake (up to 22%) would be economically efficient by reducing about 24% of the feed formulation cost without changing the nutritional quality.

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