



A study on growth performance of Nile tilapia (*Oreochromis niloticus*) by substituting fish meal with soybean meal in diet

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ABSTRACT

Replacement of fish meal with soybean meal is an effective way to relieve the pressure on fish meal as the supply of this feed ingredient is dwindling and certainly is not sustainable in long term at present levels in commercial feeds. This study was conducted to assess the growth performance (length, weight, specific growth rate) of Nile tilapia (*Oreochromis niloticus*) by replacing the percentage of fish meal with soybean meal in formulated diets. Nile tilapia (*O. niloticus*) fry were collected from Bangladesh Fisheries Research Institute, Mymensingh and treated with five formulated diets D₁, D₂, D₃, D₄, D₅ respectively containing 0%, 25%, 50%, 75% and 100% soybean meal as a replacement of fish meal. Forty *O. niloticus* having an initial weight of 6.60±0.13 g and initial length of 5.42±0.17 cm were fed these diets twice a day for 6 months. For growth analysis, length and weight of the fish were recorded at 30 days interval for 6 months. Significantly higher (P<0.001) body weight was observed in D₁ (SBM 0) and D₂ (SBM 25) groups at 90, 120, 150 and 180 days; and in D₃ (SBM 50) and D₄ (SBM 75) groups at 150 and 180 days compared to D₅ (SBM 100) group. Significantly higher (P<0.05) body length was observed in D₁ (SBM 0) and D₂ (SBM 25) groups at 180 days compared to D₅ (SBM 100) group. These results suggest that the replacement of FM up to 75% with SBM showed better growth performance in Nile tilapia (*O. niloticus*).

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Introduction

In Bangladesh aquaculture practice is expanding very rapidly due to meet the increasing demand of fish. All aquaculture practices depend on supplemental feed. Supplemental feeding is beneficial, where the production demand is high. The main protein source of supplemental feed is fish meal (FM). In most recent years, aquaculture has used approximately 46% of the total annual FM production, a figure that is expected to rise as demand for aquaculture products increases in the next decade (Miles and Chapman, 2006). The FM is a nutrient-rich ingredient commonly used in aquaculture feed. The FM contains balanced amino acid profile and high amount of energy per unit. The FM obtained from marine forage fish species has been the key protein source used in formulated feeds as a primary protein source (Miles and Chapman, 2006), particularly those fish that demand protein-rich, energy-dense diets. However, the supply of FM is dwindling and certainly is not sustainable in the long term at present levels in commercial feeds. As a result of this FM has become costly. In Bangladesh, the tannery solid wastes are converted to protein-concentrate to be used as poultry feed, fish feed, and in production of organic fertilizers

(Hossain *et al.*, 2007). One of the major concerns of protein-concentrate is the heavy metals, such as chromium, cadmium and lead. These toxic metals may accumulate in fish and pose serious health risks for consumers as hazardous waste has the possibility of directly entering the food chain. In these circumstances, a replacement of FM is urgently needed considering availability and cost effectiveness. Plant protein may be an effective replacement for FM. Many studies have designed to find alternative protein sources from plant origin, especially the grains, pulses and oilseeds (Gatlin *et al.*, 2007). Among the plant-derived alternative protein sources for FM, soybean meal (SBM) has received the highest attention due to its reasonable price, high quality, consistency and domestic availability. SBM has been known as an admirable source of protein for animals and humans (Baker, 2000).

Tilapia is the most popular species in fish farming. More than 80 countries in the world produce farm-raised tilapia. Nile tilapia (*Oreochromis niloticus*) is a fast growing, high stocking density and salinity tolerant, and easy adaptive fish. It is a delicious lean source of protein that is full of various vitamins and minerals having a

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wide range of associated health benefits. Growth assessment is a general bio-monitoring tool to evaluate the effect of the formulated diets. High growth performance was observed in tilapia by using the combinations of SBM and poultry by-product meal as replacement of FM (Koch et al., 2016). According to Zhao et al. (2010), FM could be completely replaced by soy protein concentrate without negative effect on growth in tilapia by methionine hydroxy analogue supplementation and increasing feeding frequency. Substitution of 50% of FM with SBM did not significantly impair growth performance of Japanese sea bass (Zhang et al., 2018). According to Cabral et al. (2013) plant proteins replaced up to 75% FM without impairing feed intake, growth performance and protein utilization in Senegalese sole.

In spite of having a limited FM production, a very little research has made to find a better replacement of FM till now. Continuous downfall of FM production indicates that we have to pay our attention on this matter. Therefore, a research on replacement of FM with SBM as an ingredient of fish feed in order to make steady supply of aquafeeds for aquaculture has been designed.

Materials and Methods

Site of experiment

The site of the experiment was the Mini Hatchery Complex of Department of Fisheries Biology and Genetics, Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh. Proximate analysis of feed and feed ingredient was analyzed at the Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries, BAU, Mymensingh.

Physico-chemical parameters of water

During the entire experimental period the temperature, dissolved oxygen (DO) and pH of water were monitored regularly. Water temperature of the cisterns was recorded with digital thermometer at 7 days interval. DO was determined using dissolved oxygen meter (Model: DO-5509, China) at 7 days interval. Water pH was measured by using portable digital pH meter (HI 98127, China) at 7 days interval.

Source of Nile tilapia fry and feed ingredients

Nile tilapia fry (*O. niloticus*) was a generous gift from Bangladesh Fisheries Research Institute, Mymensingh and upon arrival at the faculty were stocked in the cisterns of Mini Hatchery Complex, adjacent to the Faculty of Fisheries, BAU, Mymensingh. Continuous water circulation was provided in a closed system and maintained at 18 cm depth. Fish were offered formulated diet prepared with ingredients that were collected from Mymensingh town. Cod Liver oil was bought from local Kamal-Ranjit Market, BAU campus, Mymensingh. Feed

ingredients were used for the formulation of diet for the fish. The feed ingredients are shown in Table 1.

Analysis of the proximate composition of the feeds

After feed formulation, proximate composition of feed was analyzed to check the accuracy of the formulation. The Proximate composition of the dietary ingredients were determined following the standard methods given by Association of Official Analytical Chemists (AOAC, 2019) in the Nutrition Laboratory of Faculty of Fisheries, BAU, Mymensingh. Moisture content was determined by drying an accurately weighed amount (about 3 g) of sample in a pre-weighed porcelain in an electric oven at 105°C for 24 h. After weighing samples (about 1 g) were taken in porcelain crucibles and heated in an oven at 110°C prior to ashing at 450°C in a muffle furnace (Carbolite EML 11/6) for overnight and the average percentage of each sample of remaining material was taken as ash. Crude protein of samples was determined by following standard Micro-Kjeldhal method. Accurately weighed samples (about 0.5 g) were digested by 10 ml concentrated sulfuric acid (H₂SO₄) in presence of catalyst (Kjeldhal tablet and selenium powder) in Kjeldhal flask for about 45 min at 420°C in 2020 digester (Tecator). Then the digested material distilled with 33% sodium thiosulphate (Na₂S₂O₃) and 40% sodium hydroxide (NaOH) solution in Kjeltech system 1026 distilling unit. The distillate was collected in 25 ml of 4% boric acid (H₃BO₃) and titrated with standard hydrochloric acid (HCl, 0.2 N) and the nitrogen value obtained was converted into percentage of crude protein multiplying with a factor of 5.85. Lipid content was determined by extracting a weighed quantity of samples with analytical grade acetone in an all ground-joined Soxhlet apparatus. Accurate weighed sample (about 2.3 g) was taken in pre-weighed thimbles and dipped in acetone. Extraction was allowed to continue by heating in Electromantle ME at 70°C until clear acetone (without oil) was seen in siphon, which took about 3 h then the round bottom flask of the apparatus was separated and left for evaporation. After the evaporation of acetone, only the lipid was left in the flask that was later calculated in percentage. Accurately weighed sample (about 0.5 g) was taken in pre-weighed filter crucible and crude fibre was determined following standard procedure and finally calculated in percentage. Nitrogen free extracts was estimated as soluble carbohydrate by subtracting the sum of moisture, crude protein, ash and crude fibre from 100 and was calculated using the following formula:
NFE= 100- (moisture + crude protein + lipid + ash + crude fibre)

Feeding and growth observation of tilapia

The formulated diet was allowed to store in a freezer for later use. Formulated diet was provided to fish twice daily, once in the early morning and next at afternoon.

Effects of soybean meal on tilapia

Five diets were formulated as D₁, D₂, D₃, D₄, D₅ containing 0%, 25%, 50%, 75%, 100% of SBM as a replacement of FM. Feeding trial was continued for 6 months and length and weight of fish were observed at 30 days intervals.

Evaluation of growth performance

The variables of growth performance were calculated as follows:

Weight gain (WG; g) = $W_f - W_i$

Specific growth rate (SGR; % d⁻¹) = $(\ln W_f - \ln W_i) \times 100$

Hepatosomatic index (HSI; %) = $\text{weight of liver (g)} / W_t \times 100$

Viscerosomatic index (VSI; %) = $\text{weight of viscera (g)} / W_t \times 100$

Where W_i and W_f are the initial and final body weights (g) at the beginning and end of the feeding trial, respectively; and W_t indicates total weight of fish.

Statistical analysis

Waller Duncan multiple test range as post-hoc were performed considering a 5 % significant level by using SPSS ver. 11.5 computer software program.

Results

Water quality parameters

The water quality parameters such as temperature, pH and dissolved oxygen of cistern were 25.5±1.5°C, 7.3±0.10 and 8.5±0.5 ppm, respectively.

Proximate composition of the feed ingredients and feeds

Proximate composition of different feed ingredients and feeds was analyzed after feed formulation. Proximate composition of different feed ingredients and formulated diets is shown in the Table 2 and Table 3, respectively.

Growth performances

A significantly higher (P<0.001) mean body weight was observed in fish fed with diets D₁ (SBM 0) and D₂ (SBM 25) at 90 days, 120 days, 150 days and 180 days; and in the case of diets D₃ (SBM 50) and D₄ (SBM 75) at 150 days and 180 days compared to fed with D₅ (SBM 100) diet (Table 4). At the day of 90 and 120, significantly higher (P<0.01) final body weight was also found in fish fed with the diets D₃ (SBM 50); in case of the diet D₄ (SBM 75) significantly (P<0.05) greater weight gain was observed compared to D₅ (SBM 100) (Table 4). In D₁ group final body length was significantly higher (P<0.05) at the days of 150 and 180 (P<0.01) compared to D₅. Significantly highest (P<0.05) weight gain and specific growth rate were shown by the fish fed D₁ (SBM 0), D₂ (SBM 25), D₃ (SBM 50), D₄ (SBM 75) diets (Table 5) compared to D₅ (SBM 100). Fish fed D₅ (SBM 100) exhibited lowest (P<0.05; P<0.01; and P<0.001) final body weight, final body length, weight gain and specific growth rate whereas fish fed other diets had significantly highest ((P<0.05; P<0.01; and P<0.001)) growth rate (Table 5). It is resulted that the increased level of FM substitution had a negative effect on growth performances in *O. niloticus* but increasing FM replacement level to 75% resulted in significantly increased growth performances. HSI and VSI were not affected by the formulated diet (Table 5).

Table 1. Formulation of supplemental diet with different ingredients

Ingredients	g/kg				
	D ₁	D ₂	D ₃	D ₄	D ₅
Wheat flour	100	100	100	100	100
Wheat bran	150	150	150	150	150
Rice bran	200	200	200	200	200
Maize meal	135	135	135	135	135
Fishmeal	400	300	200	100	0
Soybean meal	0	100	200	300	400
Vitamin B	5	5	5	5	5
Cod liver oil	10	10	10	10	10

D₁, D₂, D₃, D₄ and D₅ indicate diets containing 0, 25, 50, 75 and 100% of SBM, respectively.

Table 2: Proximate composition of feed ingredients

Feed ingredients	%					
	Moisture	Crude lipid	Crude protein	Ash	Crude fiber	Carbohydrate
Wheat flour	15.51	2.20	9.22	1.68	0.8	73.59
Rice bran	18.78	10.60	11.98	7.36	7.2	44.08
Wheat bran	14.96	4.80	14.51	2.89	6.9	55.94
Maize meal	14.33	3.20	16.08	7.64	6.7	52.05
Soybean meal	14.80	4.28	41.54	5.54	6.88	26.96
Fish meal	10.17	8.46	60.61	18.03	1.8	0.93

Table 3. Proximate composition of formulated diets

Diets	%					
	Moisture	Crude lipid	Crude protein	Ash	Crude fiber	Carbohydrate
D ₁ (SBM0)	48.36	3.2	15.05	3.77	4.2	25.42
D ₂ (SBM25)	49.62	3.36	16.04	5.2	4.48	21.3
D ₃ (SBM50)	46.33	3.46	19.02	6.89	4.66	19.64
D ₄ (SBM75)	44.83	3.88	19.44	8.11	5.2	18.54
D ₅ (SBM100)	47.43	3.96	20.07	10.63	4.36	13.55

Note: SBM indicates soybean meal

Table 4: Mean weight (gm) ± SD of *O. niloticus* fed the formulated diet for 6

Diets	Intervals						
	Initial	30 days	60 days	90 days	120 days	150 days	180 days
D ₁	6.52±0.69	10.32±1.09	24.43±7.60	44.8±5.76**	62.2±6.83***	74.8±14.31***	91.2±11.99***
D ₂	6.58±0.45	10.674±1.29	26.8±7.76	46.6±2.41**	65±10.08***	80±9.51***	94±7.04***
D ₃	6.5±0.96	10.346±0.87	23.4±6.73	40±5.79**	55±7.52**	74.4±12.18***	89.8±13.99***
D ₄	6.6±0.85	9.168±0.47	19.4±3.65	35.8±2.95*	44.2±6.50*	66.4±5.59***	88±9.97***
D ₅	6.82±0.67	8.08±0.24	13.6±4.51	18.6±3.65	25.6±5.43	33.4±2.97	48.8±3.27

Values are presented as mean ± SE of triplicate samples. Values with star marks in each row are significantly different (*P<0.05, **P<0.05 and ***P<0.01 vs D₅)

Table 5: Growth performances in *O. niloticus* fed with test diets having different soybean meal replacement levels for 6 months

Parameters	Diets				
	D ₁	D ₂	D ₃	D ₄	D ₅
Initial weight(g)	6.52±0.69	6.58±0.45	6.5±0.96	6.6±0.85	6.82±0.665
Final weight(g)	89±13.99	94±7.04	91.2±11.99	88±9.97	48.8±3.271
Weight gain(g)	83.28±14.51*	87.42±6.72*	84.7±12.47*	81.4±10.75*	41.98±3.06
SGR(% d ⁻¹)	1.06±0.04*	1.08±0.02*	1.07±0.03*	1.05±0.03*	0.46±0.02
HSI(%)	1.5±0.11	1.3±0.04	1.8±0.17	1.3±0.009	1.9±0.598
VSI (%)	3.2±0.23	3.8±1.24	3.7±0.64	5.1±2.26	3.8±0.64

Values are presented as mean ± SE of triplicate samples. Values with star marks in each row are significantly different (*P<0.05 vs D₅)

Discussion

This research shows that using SBM as a replacement up to 75% of the FM in *O. niloticus* diet can give good growth. The replacement of 100% FM with SBM led to a decreased growth in fish. The greater SBM content led to lower growth rates. The fish group fed the diet with 75% SBM as a replacement of FM showed increased length and weight over the entire 180 days trial period compared to the fish fed diets with 100% SBM. According to Cabral et al. (2013), plant proteins can be reached up to 75% FM without impairing feed intake, growth performance and protein utilization in Senegalese sole. Ward et al. (2016) concluded that the group of fish fed with the diet having 60% SBM exhibited overall increased length and weight by the lowest amount over the entire 8 week trial in summer flounder (*Paralichthys dentatus*) compared to the fish fed diets with lower SBM inclusion levels. Fish growth depends on the presence of optimum protein levels in

fish feed (Khan et al., 1998; Mollah and Hossain, 1997; Mollah and Hossain, 1994). Rumsey et al., (1994) and Refstie et al. (1998) determined that high levels of replacement of FM by SBM leads to reduced growth and enteritis in salmonids. The decrease in growth from diets where SBM replaces FM at high levels in carnivorous fish may occur due to the impact of many anti-nutritional factors present in SBM, which may prevent digestion and nutrient absorption, and therefore result in higher feed conversion ratios (Grisdale-Helland et al., 2008). These include protease inhibitors (trypsin inhibitors), oligosaccharides (stachyose, raffinose, etc.), saponins, isoflavones, antigens (glycinin, conglycinin, lectins), phytate, and tannins (Refstie et al., 2006; Knudsen et al., 2007; Iwashita et al., 2009). Lin and Luo (2011) stated that the effects of FM substitution with SBM on hybrid tilapia (*Oreochromis niloticus* × *O. aureus*). Their findings showed significantly increased feed intake in hybrid tilapia. Similarly, significant enhancements in feed intake have been reported in sharp

snout sea bream (*Diplodus puntazzo*) (Hernández *et al.*, 2007) when the feed offered to the fish contain the combination of FM and SBM. Furthermore, Jahan *et al.* (2019) reported that the replacing 50% of FM with SM significantly increased muscle fibre generation of *Barbonymus gonionotus*.

Conclusion

The findings in this study showed that replacing up to 75% of FM with SBM exerted a positive effect on growth performances of *O. niloticus*.

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