

EFFECTS OF PARTIAL SUBSTITUTION OF FISHMEAL BY SOYBEAN MEAL IN NILE TILAPIA (*Oreochromis niloticus*) DIET

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ABSTRACT

The experimental trial was conducted in saline water to determine the replacement level of fish meal with locally available plant protein source, the soybean meal on growth performance, survival and body composition of Nile Tilapia (*Oreochromis niloticus*) juveniles reared in floating net cages. Four types of diet (35% crude protein) were formulated where fishmeal was replaced by soybean meal, partially up to 0%, 25%, 50% and 75% designated as treatment T1, T2, T3 and T4 respectively. The diet T1 was the control diet where a fish meal was the main ingredient and sole source of protein. A triplicate group of thirty healthy fish, juveniles (15.58g average initial weight) were stocked in each floating net cage (1.2 x 1.2 x 1.2m). Feeding was done at the rate of apparent visual satiation twice daily for a period of six weeks. At the end of the trial results indicates that there were no significant differences ($P>0.05$) in percent weight gain (%), specific growth rate (SGR %), average daily weight gain (g/ind/day), and feed conversion ratio (FCR) up to 50% fishmeal replacement by comparing with the control diet without soybean meal. However, deterioration in growth performance was noted in fish receiving more than 50% dietary soybean meal. Feed conversion ratio was poorer by increasing the soybean meal over 50%, T4 (2.81±0.74) and T3, (2.55±0.36), which is significantly different from T1 (2.21±0.12) and T2 (2.34±0.34). Survival was 94.99% in all the treatment groups. No significant differences ($P>0.05$) were found in fish final body carcass composition of protein, ash and moisture contents, but, the lipid level of the fish's body increased ($P<0.05$) significantly with an increasing fish oil and soybean meal in the diets. As a result, 50% soybean meal in the diet can substitute fishmeal without jeopardizing the growth and health condition of Nile tilapia.

Key words: Nile tilapia, Fish meal, Soybean meal, Growth performance, Floating net cages.

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INTRODUCTION

Tilapias are the second most popular cultured fishes in the world after carps (Zhou *et al.*, 2015) China, Egypt, Indonesia, Philippines and Thailand are the major producing countries (FAO, 2009). Tilapia is the excellent candidate for aquaculture because they have a high reproductive rate, resistance to diseases, rapid growth, tolerance to high stocking densities; good meat quality and reasonable price in the markets (FAO, 2011). Tilapia can be cultured in every aquatic environment like fresh water, brackish and saline water, some species of tilapia can tolerate up to 120‰ water salinity (El-Sayed, 2006). In many tropical /sub-tropical region worldwide tilapia has gained great culture potential (Ng and Hanim, 2007). Nile tilapia (*O. Niloticus*) gained important attraction for the aquaculturist and farmers due to the short food chain, fast growth rate, disease resistance, easily acceptance of artificial feed, tolerance to a variety of environmental

conditions, good quality of table food and reasonable market price (Gibtan, *et al.*, 2008).

One of the major problems facing the aquaculture industries is the fish feed. Fishmeal is conventionally used as the chief protein source in the diet of many fishes having essential fatty acids and digestible amino acids (Zhou *et al.*, 2011). About 30 – 60% of the production cost share the fish feed in net cages (Ahmad *et al.*, 2018; Silva, *et al.*, 2007). Increase in dietary fish meal protein not only increase production cost, but also reduce the growth rate in many species (Kpogue *et al.*, 2013; Ahmad *et al.*, 2019). Therefore, from both fish growth perspectives and economics the addition of protein (fish meal) in the diet should be on the optimal level (Siddiqui and Khan, 2009). The only one way to minimize this cost is to replace fish meal, partially or totally with less expensive terrestrial animal or plant proteins. The increasing demand of fish meal for high level of aquaculture activities and high price in markets

the aquaculture specialist diverts to use the alternative source of protein as compared to fish meal (Peres and Oliva-Teles, 2005).

Among the alternative plant protein sources soybean meal is an essential source of protein to replace fish meal successfully for many species, because they have a high level of digestibility, easily available in the market and reasonable price too (Lemos *et al.*, 2000). Nonetheless, the replacement level is varied species wise and depends mainly on feeding behaviors and size of fish (Sánchez-Lozano *et al.*, 2009). Dietary inclusion of soybean meal has been investigated by many researchers as an alternative to fish meal for various commercially important culture fishes like Red drum (McGoogan and Gatlin 1997), Atlantic salmon (Refstie *et al.*, 1998), Sharp snout sea bream (Rondan *et al.*, 2004), Asian seabass (Tantikitti *et al.*, 2005), Red sea bream (Biswas *et al.*, 2007), Gilthead sea bream (Bonaldo *et al.*, 2008), and Black sea turbot (Ergün *et al.*, 2008a, b; Yigit *et al.*, 2010). Although the replacement level of soybean meal for fish diets essentials to be evaluated, because soybean is known to contain some anti-nutritional factors (ANFs) like lectin and protease inhibitors, which impair the growth rate of some fish species (Azarm and Lee, 2014).

Keeping in view the high price of fish meal and reduction in the wild-captured fishes, the present experiment was planned to study the effect of possible levels of fish meal replacement with locally available plant by-product, the soybean meal on feed utilization, growth performance, body composition and survivals of Nile tilapia reared in seawater net cages.

MATERIALS AND METHODS

Feed formulation and preparation: The ingredients like fishmeal, soybean meal, wheat bran, rice bran, wheat meal and fish oil were collected from the local market. The major protein sources used in the diets were fish meal and soybean meal. The collected dry ingredients were weighed carefully and mixed together manually for five minutes, according to the formula g/100g (Table1) containing 35% crude protein level in all the four types of diets. Some water and fish oil were gradually added to the grounded particles until stiff dough was obtained. The well mixed dough was then put in a pelletizer machine for the preparation of pellets (2.5mm). The pellets were then allowed to dry in the oven for 24 hours in 45 °C to reduce the moisture of the diet. When the pellets were fully dried, they were packed in sterilized polyethylene bags and kept in freezers up to -20° C until use. After that the diets were analyzed for crude protein, crude lipids, moisture, and crud fiber contents. The proximate analysis and formulation of the prepared diets are given in Table 1. Proximate analyses were done by the process of AOAC, 1990. All the four types of diets had same physical characteristics, i.e. pellets size, color light

brown, breakable by hand, and floating capacity was approximately 2 to 4 minutes to reach the cage bottom.

Experimental fish and culture, environment: The juveniles of Nile tilapia were collected from the wild near the vicinity of experimental site 24°48'16.6"N 67°12'04.3"E. The collected juveniles were stocked in four floating net cages for two weeks in order to acclimatize. In the acclimation period fishes were fed with normal fish meal diet, and train to accept the formulated diets. At the beginning of the experiment thirty juveniles/cage in good health and active condition (15.58g, initial average weight) were randomly distributed to 1.2 x 1.2 x 1.2 m, cages four treatment groups and a triplicates for each group (30 x 12). The feed was supplied twice daily at morning 9.00 am and evening 4.00 pm by hand to apparent satiation. Fish weight and length were measured fortnightly. Water quality parameters were monitored by various methods like the temperature (°C), was by the thermometer, dissolved oxygen (mg/L) by the electronic device oxygen meter, pH by pH meter, and salinity (‰) by hand held refractometer.

Experimental fish analysis and growth performance: The growth parameter was calculated by using the following formulas; according to Sahinyilmaz and Yigit (2017, 2018), and Yigit *et al.*, 2018;

Feed Conversion ratio (FCR) = Feed intake (g) / weight gain (g)

Percentage of weight gain (%) = [(Average final weight–Average initial weight) / Average initial weight] ×100

Average daily gain (g/ind/day) = (Average final weight–Average initial weight) / Total cultured day

Survival rate (%) = (Total final number of fish/ Total Initial number of fish) × 100

Specific growth rate = % increase in body weight per day = [(ln Total final weight–ln Total initial weight) /days] × 100

At the end of the trial the experimental fishes were separately counted, measured in length and weighed individually. Five fish samples from each cage were selected for proximate whole body subsequent analysis. The selected samples were first frozen in refrigerator at -20 °C. Fish body, crude protein, moisture, Ash and crude lipids, were determined by standard methods of the Association of Official Analytical Chemists (AOAC, 1990). Crude protein (N x 6.25) was analyzed by the Kjeldahl method and crude fat by the ether extraction using the Soxhlet method. The moisture content was measured by oven drying at 105°C for 24h to extract the water content of the sample, and ash was determined by

combustion of the sample at 550 °C in a furnace for six hours.

The obtained result was presented as mean \pm standard deviation (SD) and was statistically analyzed to one way ANOVA (the study of variation) with four treatments and a triplicate per treatment using the software SPSS version 18 and Microsoft Excel 2010. The Tukey's HSD test was used to determine the differences among the treatments and the level of significance at $P < 0.05$.

RESULTS

The physico-chemical parameters including temperature, PH of water and salinity were within the acceptable range, however the dissolved oxygen (DO, mg/L) level was dropped at low tide. Water temperature did not fluctuate more than one to three degrees among the whole trial period. The water temperature ranged from 25.5 to 28.50 °C with a mean of 27.25 °C. Dissolved oxygen remained within the range of 3.5 to 6.0 ml/L (mean 4.7 ml/L). PH was around 7.1 to 8.2 (mean 7.65). Salinity was between 36.10 to 40.8‰ (mean 38.45‰) throughout the trial period. The Secchi disk visibility varied from 0.5 to 3.0 m. The four types of formulated diets were well accepted by the juveniles of Nile tilapia and survivals were not affected by increasing the soybean meal in the diets. At the end of the feeding trial all fishes were noted in healthy condition without detection of any type of disease. The growth performance parameters like average final body weight, specific growth rate (SGR), percentage of weight gain (%), average daily weight gain

(g/ind/days) and feed utilization criteria such as feed conversion ratio (FCR) are shown in Table 2. There were no significant differences detected in final weight gain, percent weight gain and specific growth rate of juvenile Nile tilapia fed at 25% and 50% soybean meal inclusion compared to the control diet.

When increasing the dietary substitution level of soybean meal over 50% a gradual decrease was observed in growth performance compared to the control diet as well as the 25% and 50% soybean meal diets. The lowest growth rate was recorded in fish fed with 75% soybean meal incorporated diet, which was significantly lower ($P < 0.05$) than the other experimental groups. Feed conversion ratio increased with the increase of the dietary soybean meal levels. The best FCR value (2.21 ± 0.12) was observed in the diet T1 without soybean meal inclusion followed by T2, 25% soybean meal (2.34 ± 0.3) and T3, 50% soybean meal (2.55 ± 0.3). A poorer FCR value of 2.81 ± 0.74 was recorded in the T4, group with 75% soybean meal incorporation. The final weight gain against varying soybean meal in the diets is displayed in Fig. 1. The final weight gain was more or less similar in the fish fed soybean meal diets at 0%, 25% and 50%, but the diet with 75% soybean meal addition resulted in significantly lower weight gain at the end of feeding experimental trial. Specific growth rate was decreased by increasing dietary soybean meal over 50%, while the highest rate of SGR (2.04 ± 0.038) was obtained in fish fed 0 % soybean meal diet followed by 25% (1.96 ± 0.19), 50% (1.89 ± 0.19) and 75 % (1.72 ± 0.52) soybean meal diet respectively.

Table 1. Feed formulation (g/100) and chemical composition (%) of the experimental diets for Nile tilapia.

Ingredients	Experimental Diets			
	T1 0% SBM	T2 25% SBM	T3 50% SBM	T4 75% SBM
Fish meal	30	24.1	18.2	12
Soybean meal	0	6.2	12.1	18.5
Wheat meal	16	15.2	14.7	14
Rise meal	15	15	15	15
Wheat bran	30	30	30	30
Alpha Starch	6	6	6	6
Fish Oil	1	1.5	2	2.5
Vitamins & Minerals	2	2	2	2
Chemical Composition				
Dry Matter (%)	88	89	90	87
Moisture (%)	9.8	10.1	9.8	9.7
Crude Protein (%)	35.2	35.1	34.8	35.2
Crude Fat (%)	5.2	5.3	5.7	5.2
Crude Fiber (%)	6.8	5.9	6.7	6.5

Fish meal (61.2% CP), Soybean meal (SBM) (45.3% CP), Wheat meal, (16.2% CP), Wheat bran (12.6%, CP), Rice bran, (6.1%, CP), Starch. (9.4%, CP), CP represents crude protein.

The whole body proximate compositions are shown in Table 3. The carcass composition of fish final body protein level was not influenced significantly by increasing the level of dietary soybean meal. The ash content and moisture level of fish fed soybean meal

incorporated diets at finally remained similar to the group fed with the control diet, and no significant difference ($P>0.05$) was observed, however the lipid content of Nile tilapia increased significantly by increasing the soybean meal as well as fish oil in the diets.

Table 2. The growth performance of Nile tilapia fed on control (fishmeal diet) and different levels of fishmeal replacement by soybean meal.

S. No.	Parameters	T1	T2	T3	T4
		0% SBM	25% SBM	50%SBM	75% SBM
1	Average initial weight	15.37 ±0.212	15.64±1.385	15.57±1.364	15.75±1.21
2	Average final weight gain (g)	36.21±1.110	35.64±0.141	34.54±0.106	32.53±1.51
3	Percentage of weight gain (%)	135.58±3.981	127.87±4.21	121.83±5.44	106.53±4.25
4	Average daily weight gain (g/ind/day)	0.49±0.014	0.47±0.028	0.45±0.035	0.39±0.63
5	Feed conversion ratio (FCR)	2.21±0.12	2.34±0.34	2.55±0.36	2.81±0.74
6	Specific growth rate (SGR)	2.04±0.038 ^a	1.96±0.197 ^a	1.89±0.197 ^{ab}	1.72±0.52 ^c
7	Survival rate %	96.66±4.71	93.33±4.71	93.33±4.71	96.66±4.71

Mean ± SD of triplicate group of 30 fishes: ^a ^b ^c significance differences ($P<0.05$) in the same row with different letter.

Table 3. Initial and final whole body proximate composition (dry basis) of juveniles *Oreochromis niloticus* before and after the feeding experiment.

Composition	Initial	Diets			
		T1, 0% SBM	T2, 25% SBM	T3, 50% SBM	T4, 75% SBM
Moister (%)	10.12	10.83	10.92	11.32	11.25
Protein (%)	60.14	68.12	69.21	68.34	69.12
Lipids (%)	16.13	16.22 ^a	17.32 ^a	20.15 ^b	21.62 ^b
Ash (%)	13.18	12.34	12.82	13.33	13.12

Values are means of triplicate group's ± SD. Within a row, means with the same letters are not significantly different ($P<0.05$).

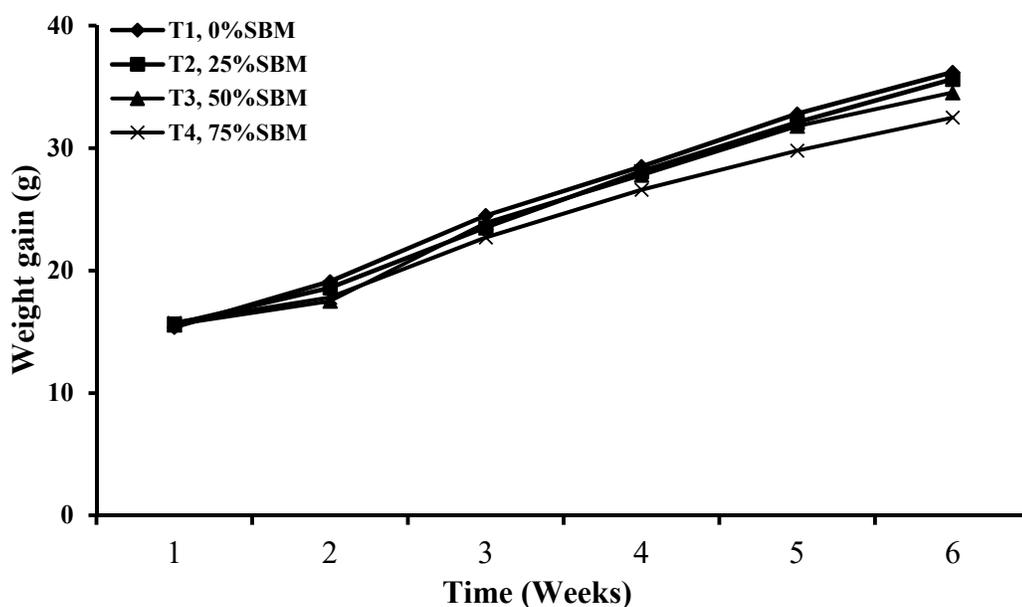


Figure 1. Weight gain (g) of Nile Tilapia offered four test diets, where fishmeal were replaced with soybean meal (SBM) (T1, 0%, T2, 25%, T3, 50%, T4, 75%) during the experimental period.

DISCUSSION

There has been an increasing attention towards cost-effective diets and practical attitudes for efficient use of alternative of protein source, especially in marine fish species for sustainable aquaculture practices (Kader *et al.*, 2012). Hence the present experiment was designed to determine the fish meal replacement level with soybean meal to examine the growth performance of Nile tilapia in saline water net cage systems. The results of the present finding observed that the inclusion level of soybean meal in Nile tilapia up to 50% did not affect growth performance significantly compared to the control diet, which might be due to the similarities of the amino acid composition of soybean meal with fish meal. This is in covenant with some previous findings on different fish species. For example Yatawaraandm, and Hettiarachchi (2006) report that fish meal can replace by heat processed soybean meal in the diet of Nile tilapia up to 50% with no negative effect on growth rate. The Nile tilapia, (*O. niloticus*), can give a better results by replacing fish meal with feather meal up to 50%, while a mixture of feather meal could be replaced up to 35% of the fish meal without a decrease in growth rate (Higgs *et al.*, 1982). Similarly, many authors reported that fish meal can be substituted by soybean meal up to 75% and get satisfactory result for Nile tilapia (EI-Sayad, 1998). Results from earlier reports on tilapia are in agreement with those of other fish species such as gilthead sea bream (*Sparus aurata*) or rainbow trout (*Oncorhynchus mykiss*) fed soybean meal incorporated diets up to 40% up to 40% (Venou *et al.*, 2006; Harlioglu, 2011), sharpnose seabream (*Diplodus puntazzo*) up to 60% (Hernandez *et al.*, 2007), red drum (*Sciaenops ocellatus*) up to 90% (McGoogan and Gatlin, 1997), Black Sea turbot (*Psetta maxima*) up to 20 % (Ergün *et al.*, 2008a,b; Yigit *et al.*, 2010). The blue catfish tolerates replacement of fish meal up to 100% with methionine supplementation and no adverse effect on growth and fish body (Webster *et al.*, 1995). Such type of result is shown by Bonaldo *et al.*, (2008) for European sea bass and gilthead seabream also (Bonaldo *et al.*, 2008).

In the present study protein, moisture and ash contents in fish body were not influenced significantly by the increase of dietary soybean meal; however a significant change in lipid content was noted in Nile tilapia. The increase of lipid level in fish body might be due to increasing level of full fat soybean meal and fish oil in the diet. Similar results have previously been reported for gilthead seabream (*Sparus aurata*) and Atlantic salmon (*Salmo salar*) (Refstie *et al.*, 2001; Martínez-Llorens *et al.*, 2007; Bonaldo *et al.*, 2008). The dissolved oxygen level in the area was dropped in to 3.5mg/L at low tide where the mortality was recorded in sensitive fish groups, like, sea breams, anchovies and mullets at low tide on the other hand the Nile tilapia was

survived at low tide and low dissolved oxygen level in the area because the Nile tilapia is a hardy fish and can tolerate the low level of dissolved oxygen. That's why the installed cages area is suitable only for Nile tilapia culture.

In conclusion, the results of the present study based on feed utilization and growth performance suggest that 50 % soybean meal can be included to replace fish meal in the diets of Nile tilapia (*O. niloticus*) with no negative effects on growth and body composition when cultured in floating net cage systems in a marine environment.

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