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Effect of Replacement of Soybean Meal with Sesame Meal in The Diet of Thai-Chitralada Strain of *Oreochromis niloticus* (L)

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ABSTRACT

Background: The present study was aimed to determine effect of replacement of soybean meal (SBM) with sesame meal (SSM) in the diet of Juvenile Thai chitralada tilapia.

Methods: The study was undertaken with different inclusion levels of sesame meal (SSM) such as T1 (25% SSM), T2 (30% SSM), T3 (35% SSM) and control feed (C) for a period of 90 days.

Result: Thai-Chitralada tilapia fed with T3 (35% SSM) diet attained maximum mean weight gain (28.09 g), highest specific growth rate (SGR) (2.69%), best feed conversion ratio (FCR) (1.04), maximum average daily growth (ADG) (0.31 g) and maximum protein efficiency ratio (PER) (8.87). The lowest growth rate (GR) was observed in the group fed with control diet. Diets with 35% SSM replacement upon SBM showed higher amino acid (AA) profile than other treatments and control diet. Analysed AA profile in T3 - 35% SSM was Arg - 2.88, Hist - 0.86, Isol - 0.92, Leu - 2.60, Lys - 3.78, Met - 0.39, Cys - 0.38, Phe - 1.77, Thr - 1.08, Val - 0.99, Tyro - 1.09, Trypt - 0.24. The higher DNA / RNA ratio of Thai-Chitralada tilapia was obtained in T3 - 0.459, followed by T1 - 0.44 and T2 - 0.323. The lower DNA / RNA ratio of Thai-Chitralada was obtained in control - 0.321. One way ANOVA of the data analysis and Duncan multiple range test clearly affirmed that different between and sampling periods, Thai-Tilapia fingerlings had significant difference ($P<0.05$) among the different experimental diets. From the present experiment, it could be concluded that, sesame seed meal can replace 35% SBM in the diets of Thai-Chitralada tilapia.

Key words: Amino acid profile, Bio-growth, DNA/RNA ratio, Feed parameters, Sesame meal, Soybean meal, Thai-Chitralada tilapia.

INTRODUCTION

Fisheries and aquaculture remain as an important sources of food, nutrition, income and livelihood for coastal fisherfolks around the world. Nutrition, feed and feed management plays an important role in the development of sustainable aquaculture. SBM has been the most common source of plant protein as a fish meal replacer in aquaculture feed because of its high protein content, relatively well-balanced AA profiles, reasonable price and steady supply (El-Sayed, 1999). Therefore, the search for alternative plant proteins to replace SBM has been gained increasing interest in the research of aquaculture nutrition (Barros *et al.*, 2002). SSM is a plant protein source and used to reduce the feed costs by replacing SBM in fish diets (Tacon, 1993). Sesame seeds are the source of EAA and sulphur amino acids (Lee *et al.*, 2003). Successful and sustainable culture of finfish and shellfish depends on the use of nutritionally balanced, low-cost and eco-friendly feeds (Joseph and Ignatius, 2016). Dietary nutrient requirements in fish are usually estimated empirically by feeding graded levels of a specific nutrient, in a basal diet containing a different level of that nutrient and then measuring growth, feed intake, body nutrient stores or other variables (Lekshmi and Prasad, 2014). RNA:DNA ratio is a frequently measured indicator of growth rate. RNA:DNA ratios were used successfully to predict growth and nutritional state in a multitude of studies on variety of organisms. The present study was aimed to determine effect of replacement of SBM with SSM of Juvenile Thai chitralada tilapia based on biogrowth parameters, DNA/RNA ratio and feed related parameters like proximate and AA profile analysis.

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MATERIALS AND METHODS

Experimental diet

Feed ingredients such as SBM, fish oil, fish hydrolysate, rice bran oil, mono calcium phosphate, vitamin and mineral premix were obtained from Growel feeds Pvt, Limited, Andhra Pradesh, India. SSM, common salt and cassava starch were purchased from local market. The ingredients were dried well and powdered. These major ingredients were mixed in the feed at three different concentration *viz.,* SSM 25%, 30% and 35%. The control feed was prepared without adding SSM. All the ingredients and feed additives except vitamin and mineral mixture were mixed well and made it as a ball and cooked in a pressure cooker for 10-15 minutes. Then each dough was pelletized by using the manual pelletizer. Then pelletized feeds were dried at 60°C for 12 h and stored in airtight containers. The ingredients composition of control feed and experimental diets and proximate composition of protein supplements were presented in Table 1 and 2**.**

Proximate analysis and Amino acid analysis of experimental feed

The proximate analysis of experimental feed was estimated using standard method (AOAC, 1995). The results were given in Table 3. The amino acid profile of the experimental feed were given in Table 4.

Experimental setup

The experimental set up comprised of three sets of treatments and one sets of control (Replicates) for a period of 90 days. Experiment was conducted in rectangular cement tanks (volume: 1000 l) installed with 14 fish hapa's of the size 0.30 m^3 (0.73 m*0.59 m*0.71 m) (volume: 300 l) were used.

Experimental fishes

The experimental fish (Thai-Chitralada) were procured from Svara Biotechnovations fish farm, Madurai, Tamil Nadu. All the fish seeds were properly acclimatized in cement tanks. The fishes were graded according to their weight prior to the experiment. An average of 2.650 g size 460 Nos of Thai-Chitralada were selected for the experiment. The fishes were stocked at 30 numbers per hapa and capacity of the hapa is 300 l. Daily the fishes were fed @ 5% of their body weight. Fortnightly the sampling was carried out. The bio-growth parameters were calculated.

DNA/RNA ratio

Estimation of RNA and DNA in fish were done by method of Buckley and Bulow (1987) and the results were presented in Table 5.

Extraction of nucleic acids

In Laboratory, larvae were thawed and measured (0.1 mm) under a dissecting microscope equipped with ocular

Table 1: Ingredient composition of experimental feed.

Ingredients	Control feed	SSM 25	SSM 30	SSM 35
Soybean meal	60.70	35.70	30.70	25.70
Sesame seed meal	0.00	25.00	30.00	35.00
Cassava starch	27.20	27.20	27.20	27.20
Rice bran oil	4.40	4.40	4.40	4.40
Fish oil	1.00	1.00	1.00	1.00
Fish hydrolysate	3.00	3.00	3.00	3.00
Mono calcium phosphate	1.70	1.70	1.70	1.70
Vitamin premix	0.50	0.50	0.50	0.50
Mineral premix	0.50	0.50	0.50	0.50
Common salt	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00

Table 2: Proximate composition of protein supplements (dry matter basis).

Constituent	Soybean meal	Sesame meal
Moisture (%)	7.14	6.21
Crude protein (%)	51.94	48.32
Crude fibre (%)	3.24	7.30
Ether extract (%)	1.39	1.02
Total ash (%)	7.21	6.1
Gross energy(Kcal/Kg) = 5.7*g protein+9.4*g fat+4.1*(gNFE+g fibre)	4392	4405

Table 3: Proximate composition of SSM (dry matter).

Diet	Moisture (%)	Crude protein (%)	Crude fibre (%)	Ether extract (%)	Total ash (%)	Gross energy (Kcal/Kg)
Control	7.08	31.00	1.23	3.91	7.54	4058
SSM 25	8.02	31.50	1.25	3.78	6.51	4063
SSM 30	8.05	31.28	1.46	3.98	6.98	4059
SSM 35	7.53	31.75	2.90	4.08	7.22	4086

Table 4: Amino acid analysis of experimental diets.

Diet	Arg	Hist	Isol	Leu	Lys	Met	Cys	Phe	Thr	Val	Tyro	Trypto
Control	2.34	0.70	0.85	2.01	2.36	0.13	0.35	1.64	1.06	0.78	0.24	0.20
T1 - 25% SSM	2.75	0.82	0.88	2.51	3.36	0.38	0.38	1.76	1.08	0.96	1.07	0.29
T2 - 30% SSM	2.64	0.73	0.86	2.41	3.11	0.22	0.36	1.70	1.07	0.93	0.93	0.24
T3 - 35% SSM	2.88	0.86	0.92	2.60	3.78	0.39	0.38	1.77	1.24	0.99	1.09	0.30

Table 5: DNA / RNA ratio of Thai-Chitralada tilapia (Sesame meal incorporated diet).

DNA / RNA ratio	Control	T1 (25 SSM)	T2 (30 SSM)	T3 (35 SSM)
45th day	0.057± 0.00^{b}	0.0819± 0.01^{a}	0.060± 0.00^{b}	0.158± 0.00^{b}
90th day	0.321^{cd}	0.442± 0.01 ^{b}	0.323± 0.02^{c}	0.459 ±0.03^{a}

Values in the same rows with different superscript different significantly (P<0.05) for each parameters. One way ANOVA was used following Duncan multiple ranges testing SPSS 16.0. Values are presented as mean ± Standard error.

micrometer. Conventional fluorimetric analysis (CFA) developed by Clemmesen (1988, 1993) and further modified by Chicharo (1996) modified ´ fluorimetric analysis (MFA) were used to quantify nucleic acids in individual fish larvae. Fish larvae were extracted in 0.15 ml of 1% sarcosine (sodium N-lauroylsarcosine) in Tris-EDTA buffer (pH 8.0) to give a final concentration of 0.1%. After centrifugation, aliquots of the supernatant were used for further analysis.

CFA

A 0.2 ml aliquot of extracted sample was combined with 0.4 ml of Tris-NaCl 21 (pH 7.5) and 0.05 ml of ethidium bromide (0.1 mg/ml). Another 0.2 ml aliquot of the same extracted sample was combined with 0.35 ml of Tris-NaCl 21 and 0.05 ml of ribonuclease A (Type-II A, 0.12 mg/ml). This mixture was incubated at 37.8°C for 30 min, allowed to reach room temperature for 15 min and stained with 0.05 ml of EB.

MFA

For purification of nucleic acids, a third 0.6 ml aliquot of the extracted sample was washed with 0.6 ml of phenol-chloroform-isoamyl alcohol (49.5:49.5:1, v/v) and then with 0.3 ml of chloroform-isoamyl alcohol (24:1, v/v). After these purification steps, 0.2 ml aliquots of the supernatant were treated as above for CFA.

Fluorescence assays

Calculations of nucleic acids concentration were identical for both procedures. Endogenous sample fluorescence (blank) was subtracted from total sample-EB dye fluorescence. The fluorescence due to total RNA, mainly ribosomal, was calculated as the difference between total fluorescence (RNA and DNA) and the fluorescence measured after ribonuclease treatment, which is assumed to be due to DNA. Fluorescence was determined by exciting at 365 nm and reading at 590 nm with a spectrofluorometer (Hitachi Model 650-10). Concentrations were determined by running standard curves of DNA-EB and RNA-EB every day with known concentrations of 21 21 l-DNA (0.25 mg/ml) and 16s-23s RNA (4 mg/ml), in the appropriate range of values. All chemicals used in the procedures described above were analytical grade. The limit of detection, *i.e.* the analyte concentration giving a signal equal to the blank signal plus 2 standard deviations of the blank (Miller and Miller, 1984), was 0.16 21 21 mg/ml for DNA and 0.46 mg/ml for RNA. Percent recovery of added l-DNA to eight larvae homogenates (DNA spike) was 95.3% for CFA and 88.8% for MFA and the recovery of added 16s 1 23s RNA (RNA spike) was 105.6% for CFA and 62.8% for MFA. Total amounts of nucleic acids were corrected based on these values. The coefficient of variation (Zar, 1999) calculated for estimate from eight homogenate samples was: (1) 1.5% for DNA and 3.5% for RNA when using CFA and (2) 14.8% for DNA and 17.8% for RNA when using MFA.

AA profile analysis

The AA profile of experimental diets were analyzed by Ultra Pressure Liquid Chromatography (Model- Waters ACQUITY-UPLC, Waters, Massachusetts, USA), following the method of Ishida *et al.* (1981) at TNJFU Referral Laboratory for Fish Quality Monitoring and Certification, FC and RI, Thoothukudi.

Waterquality parameters

Water quality parameters were estimated using standard procedures (APHA, 2005). The average range of water quality parameters were presented in Table 6.

Statistical analysis

All the data of this study were examined to one-way analysis of variance (ANOVA) utilizing the statistical software program SPSS version 16.0 (SPSS Inc., IL, USA). Duncan's posthoc

Table 6: Water quality parameters of Thai-Chitralada tilapia (Sesame meal incorporated diet).

Parameters	Range
Temperature	27.5-29.0°C
Dissolved Oxygen	5-6 ppm
pH	8.2-8.4
Ammonia	0.01-0.03 ppm
Nitrite	0.03-0.07 ppm
Nitrate	10-12 ppm
Hardness	365-395ppm
Alkalinity	167-172 ppm

test was used to compare the averages of data at significance level of $p<0.05$.

RESULTS AND DISCUSSION

Influence of sesame seed meal on the growth of Thai-Chitralada Tilapia

Influence of SSM on the bio growth performance of Thai-Chitralada Tilapia are given in Table 7.

The MWG of the fishes fed with SSM at 35% was 28.09±0.09[cd] g which is higher than that of the fishes fed with control feed (19.60±1.07[a]). When SGR of the fishes fed with SSM is concerned, 35% yielded best SGR (2.69±0.315[b]) rather than control and other treatments. The ADG rate of the fishes fed with 35% showed 0.31±0.00[cd] which is higher than control and other treatments. The ADG was very low in control when compared to 25%, 30% and 35% treatment fishes. The SSM at 35% yielded the best mean weight gain rather than control and other treatments. Compared with SBM, SSM has higher methionine content, but its lysine content is comparatively low. Among, three concentrations used, SSM at 35% yielded best. The BWG of SSM 35% was observed to be 28.09±0.09[cd] g which was higher than control. Growth performance and body composition of rainbow trout were not affected by replacing fish meal with SSM up to 39% (Nang Thu *et al.*, 2011). This result was compromising with our result where SSM 35% showed higher growth rate which was the highest inclusion level.

Jahanbakhshi *et al.* (2012) indicated the substitution compliance of SSM and corn gluten could be between 16-48% for Beluga *(Huso huso)* fingerlings. SSM was suggested by Tacon (1992) of its maximum level of inclusion in both omnivorous and herbivores fish species to be 35%. So it is clearly shown that SSM in this experiment gives better growth for Thai-Chitralada Tilapia which is an omnivorous. Hossain and Jauncey (1989) reported SSM can be included upto 25% in raw condition in the diet of *Cyprinus carpio* L. Hossain *et al.* (1992) substituted fish meal with 20% SSM in the diets of catfish, *Heteropneustes fossilis* and reported promising result. Olukunle and Falaye (1998) found that 25% SSM cake incorporation supported weight gains in *C. gariepinus* similar to diets with 100% fish meal. FCR was not affected by increasing ratio of SSM in diet according to the studies of Emadi *et al.* (2014) in rainbow trout and Enyidi *et al.* (2014) in African catfish. Similarly, in the present study, increasing SSM ratio up to 35 % in the diet did not affect the feed conversion ratio which was between 1.04 and 1.156.

DNA / RNA ratio

In this study the average RNA: DNA ratio of fish fed with control diet on 45[th] day was 0.057±0.00[b] in 90[th] day which was increased up to 0.321±0.04[ab]. A higher RNA: DNA ratio was recorded in T3 (35% CSM protein) diet fed fish compared to control and other treatments. Labh (2015) reported that DNA / RNA ratios were increased rapidly with age and sizes of fishes.

Results of amino acid analysis

Results of amino acid analysis of experimental diets were given in Table 4. In the present study, diets with 35% SSM replacement upon soybean meal showed higher AA profile than other treatments and control diet. From this evaluated AA profile, Methionine seems to be less than the requirement level for *Oreochromis niloticus*. (Santiago and Lovell, 1988) Plant-based materials are being investigated as alternatives and cheaper sources of protein for the replacement of the highly competitive fish meal in fish diets.

Feed cost

SSM in the fish diets could reduce the feed costs. When feed cost was taken into consideration, it revealed that those feeds

Table 7: Growth performance and feed utilization of Thai-Chitralada tilapia (Sesame meal incorporated diet).

Diet parameters	Control	T1 (25 SSM)	T2 (30 SSM)	T3 (35 SSM)
Stocking density (Nos)	30	30	30	30
Mean initial weight (g)	2.61±0.04[a]	2.58±0.06[a]	2.62±0.07[a]	2.72±0.07[a]
Mean final weight (g)	22.21±1.11[a]	27.82±0.27[b]	27.81±0.08[b]	30.82±0.02[cd]
Mean weight gain (g)	19.60±1.07[a]	25.24±0.34[b]	25.19±0.81[b]	28.09±0.09[cd]
Mean initial length (cm)	2.20±0.20[a]	2.50±0.10[a]	2.25±0.15[a]	2.30±0.10[a]
Mean final length (cm)	13.55±0.25[a]	14.85±0.25[bc]	14.35±0.5[abc]	15.15±0.5[c]
Average daily growth(g)	0.21 0.01[a]	0.28±0.00[b]	0.27±0.00[b]	0.31±0.00[cd]
Survival rate (%)	96±0.00[ab]	95±0.17[a]	96±0.00[ab]	100±0.00[b]
Specific growth rate (%/day)	2.38±0.03[a]	2.64±0.03[b]	2.62±0.00[b]	2.69±0.315[b]
Biomass(g)	189.58±10.3[a]	229.72±1.06[b]	243.59±0.83[b]	280.95±0.95[c]
Total biomass gain (g)	214.82±10.78[a]	264.29±2.11[b]	268.97±8.55[b]	308.20±0.20[cd]
Feed conversion ratio	1.15±0.08[cd]	1.10±0.04[b]	1.14±0.00[a]	1.04±0.02[a]
FCE	86.95±6.31[a]	91.03±4.08 [a]	87.33±0.38[a]	96.05±2.59[a]
Protein efficiency ratio	5.92±0.32[c]	7.74±0.03[b]	7.72±0.24[b]	8.88±0.03[a]

Values in the same rows with different superscript different significantly (P<0.05) for each parameters. One way ANOVA was used following Duncan multiple ranges testing SPSS 16.0. Values are presented as mean ± Standard error.

containing SSM in different proportions T1 - 25% SSM (Rs. 46.57), T2 - 30% SSM (Rs. 46.42), T3 - 35% SSM (Rs. 46.27) were cheaper than the control diet (Rs. 50.00).

CONCLUSION

The present study concluded that, SSM at 35% are better alternative plant protein sources to replace Soybean meal for the better growth of Thai-chitralada Tilapia which was evident from the improved growth performances with increasing concentrations, apparently high DNA/RNA ratio leads to indicate higher growth rate of the fishes when the fishes fed with 35% SSM.

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Conflict of interest

The authors declare that they have no conflict of interest.

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