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Research Article

Total Replacement of Fishmeal by Soybean Meal with or Without Methionine Fortification in the Diets of Nile Tilapia, *Oreochromis niloticus*

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Abstract

The high cost and competing demand for fish meal in fish diet has made the search for alternative ingredient very expedient. A 12 week feeding trial was conducted with *Oreochromis niloticus* to determine the effect of replacing fishmeal with soyabean meal and methionine on growth, nutrient utilization, haematology and plasma biochemistry. Four experimental diets (40% crude protein) were formulated, with Partial Soybean Meal (PSM) inclusion, No Soyabean Meal (NSM), Only Soybean Meal (OSM) and soybean meal and methionine. For 84 days, fish were fed at 3% body weight daily to triplicate groups of 15 fish (25.08 g±0.04 g fish⁻¹) that were randomly allocated to twelve hapa suspended in 20 m² earthen pond. Results showed that Mean Weight Gain (MWG) and increase in length were significantly high (p<0.05) in PSM (22.77 g and 16.90 cm, respectively) and least in NSM (17.54 g and 14.63 cm, respectively). Specific Growth Rate (SGR) values were high in PSM (1.62) and OSM+M (1.55) and least in NSM (1.48). Fish fed OSM+M had the highest Parked Cell Volume (PCV, 20.66%), haemoglobin (Hb, 7.87 g dL⁻¹) and least White Blood Cells (WBC, 3.12x10⁴ mm⁻³), while NSM gave the least Hb (5.37 g dL⁻¹) and highest WBC (3.92x10⁴ mm⁻³). Serum enzymes AST (68.0 U L⁻¹) and ALT (46.66 U L⁻¹) were high in OSM+M and least in PSM (47.66 and 28.64 U L⁻¹, respectively). The present study revealed that soybean meal can either be used to partially replace fishmeal or completely replace it with methionine supplementation, when growth, nutrient utilization and health status of *O. niloticus* are considered.

Key words: Nile tilapia, plant protein, methionine, nutrient, haematology

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nigeria is the second largest producer of farm-raised tilapia in Africa, after Egypt (Fagbenro *et al.*, 2010), however, her contribution to global output is just a meager 28,950 metric tons. Precocious breeding and high cost of feed are among the militating factors to tilapia production. Although, there has been breakthrough in checking the precocious breeding of tilapia through genetically modified parent stock and administration of Methyl-testosterone (MT) feed, availability of cost effective and highly potent feed still remains a major constraint to tilapia culture.

Protein has been described as the most expensive and important components of fish feed and its major source is fishmeal. This is because of its high protein content, well balanced essential amino acid and fatty acid profiles and given the high correlation between whole body Indispensible Amino Acid (IAA) profile and the IAA requirement pattern (Mambrini and Kaushik, 1995). Fishmeal is however implicated in the prohibitive cost of sustainable aquafeed production due to increased price, dwindling supply and competition from other users. This has necessitated increased research into alternative protein sources for fish (Ayoola, 2011). Soybean meal, which is considered as the most nutritive plant ingredient is a potential alternative protein sources for the replacement of fishmeal. However, Ologhobo (1992) reported that plant proteins are low in sulphur amino acids (methionine and cystine). Although, several studies have recorded considerable success in the partial or total replacement of fishmeal with variously processed soybean meal in the diet of Oreochromis niloticus (Al-Kenawy et al., 2008; Wu et al., 2004; Siddhuraju and Becker, 2003; Nyirenda et al., 2000) information on the effect of replacing fish meal with soybean meal and methionine at juvenile stage of O. niloticus till remains scanty. This study therefore, investigates the effect of fishmeal replacement with soybean meal with or without methionine on the growth performance, nutrient utilization and health status of Nile tilapia juveniles.

MATERIALS AND METHODS

Experimental fish and culture condition: One hundred and eighty juveniles of *Oreochromis niloticus* were obtained from a reputable fish farm in Ibadan and acclimatized for 14 days to laboratory conditions at the Teaching and Research Farm Department of Aquaculture and Fisheries Management, University of Ibadan. A total of 15 juveniles (mean weight 25.08±0.04 g and mean total length of 12.20±0.21 cm) were

randomly stocked into each hapa (1 m³) already suspended in 20 m² earthen pond. Treatments were replicated thrice. Water level was maintained at 1.2 m by pumping water into the pond weekly. Temperature and dissolved oxygen were monitored daily using a Combined Digital Probe (YSI Model 57, VWR Company, New Jersey), while a pH meter (Metler Toledo-320 model U.K). Ammonia-nitrogen and nitrite-nitrogen were determined by colorimetric methods on a weekly basis.

Feed and feeding: Four isonitrogenous diets (40% crude protein) designated as PSM, NSM, OSM and OSM+M (Partial replacement of fishmeal with soyabean meal, no soyabean meal, only soyabean meal and soyabean meal with methionine, respectively) were formulated as shown in Table 1. Ingredients were finely ground and mixed thoroughly in a pelleting machine (Hobart A200, Troy Ohio, USA). The homogenized mass was pelletized through a 2 mm diet and pellets were sun-dried and packed into labeled nylon bags for use. Diets were fed to triplicate groups of fish at 3% body weight twice daily (0900 and 1700 h) for 84 days. Feeding response was monitored and mortality recorded. The weight and standard length of fish were measured at the start of the experiment and thereafter biweekly. Fish weight and length were determined using weighing scale (OHAUS model CS 5000) and measuring board, respectively. Recorded weight was used to adjust for feed requirements biweekly.

Chemical analysis: Feed ingredients, diets and fish carcass (5 fish from pool as initial values) were analysed for proximate

Table 1: Gross and proximate composition of the experimental diets

Feed stuff	PSM	NSM	OSM	OSM+M
Maize	14.06	27.66	3.80	3.67
Wheat offal	7.70	13.93	1.90	1.89
Fish meal (65%)	23.20	49.91	-	-
Soyabean meal (42%)	46.47	-	85.80	83.93
Dicalcium phosphate	3	3	3	3
Premixes	2	2	2	2
Salt	0.5	0.5	0.5	0.5
Vegetable oil	3	3	3	3
Methionine	0.1	-	-	2.01
Total				
Analyzed composition (%)			
Crude protein	41.49	40.67	39.98	41.83
Crude fat	3.40	2.79	3.14	2.87
Ash	11.00	9.85	11.25	10.94
Moisture	12.00	8.32	8.72	8.27
Nitrogen free extract	27.31	34.79	33.60	32.50
Dry matter	91.30	91.68	91.28	91.73

PSM: Soybean meal inclusion, NSM: No soybean meal, OSM: Only soybean meal and OSM+M: Only soybean meal and methionine

Table 2: Growth performances and nutrient utilization of *Oreochromis niloticus* fed experimental diet (Mean±SD)

Parameters	PSM	NSM	OSM	OSM+M
Initial weight (g)	25.26±0.03ª	25.08±0.04 ^a	25.09±0.04 ^a	25.11±0.06 ^a
Final weight (g)	48.04±0.12 ^a	42.62±0.72 ^d	43.24±0.73°	44.98±0.19 ^b
Weight gain (g)	22.77±0.86ª	17.54±0.41 ^d	18.14±0.13°	19.87±0.18 ^b
Weight gain (%)	190.13±0.20ª	168.77±0.41 ^d	172.29±0.24°	179.12±0.72
Initial length (cm)	12.32 ± 0.17^{a}	12.20±0.21 ^a	12.21 ± 0.50^{a}	12.21±0.61 ^a
Final length (cm)	16.90±0.35ª	14.63±0.14 ^b	14.67±0.32 ^b	15.37±0.43 ^b
Feed conversion ratio	3.99±0.00°	4.83±0.01 ^a	4.76 ± 0.08^{a}	4.36±0.88 ^b
Protein efficiency ratio	0.56 ± 0.00^{a}	0.43 ± 0.00^{d}	$0.45\pm0.00^{\circ}$	0.49 ± 0.00^{b}
Specific growth rate	1.62 ± 0.00^{a}	1.48±0.00 ^b	1.49±0.07 ^b	1.55±0.01 ^b
Survival rate (%)	100.00 ± 0.00^{a}	100.00 ± 0.00^a	100.00 ± 0.00^a	99.07 ± 1.60^a

Mean with same subscript along the same row are not significantly different (p>0.05), PSM: Soybean meal inclusion, NSM: No soybean meal, OSM: Only soybean meal and OSM+M: Only soybean meal and methionine

Table 3: Initial and final proximate composition of *O. niloticus* juveniles fed experimental diets

Parameters	Initial	PSM	NSM	OSM	OSM+M
Crude protein (%)	44.03±0.00 ^a	53.27±0.14 ^b	52.79±0.05b	52.88±0.05b	53.15±0.57 ^b
Crude fat (%)	4.89 ± 0.05^{a}	6.68±0.05b	6.85±0.06 ^b	6.74±0.05 ^b	7.23±0.01 ^c
Ash (%)	9.80 ± 0.05^{a}	13.37±0.00 ^d	12.79±0.00°	12.69±0.01 ^b	13.64±0.00e
Moisture (%)	6.12±0.00e	4.89 ± 0.00^{a}	5.25±0.00d	5.13±0.00 ^c	4.94±0.00b
Nitrogen free-extract (%)	35.16±0.01e	20.48±0.00 ^b	21.04±0.00 ^c	21.21±0.03 ^d	19.74±0.03ª
Dry matter (%)	93.95±0.03°	95.04±0.07°	94.21 ± 0.26 ab	94.62±0.31bc	95.02±0.01°

Mean with same superscript along the same row are not significantly different (p>0.05), PSM: Partail soybean meal inclusion, NSM: No soybean meal, OSM: Only soybean meal and OSM+M: Only soybean meal and methionine

compositions using official methods described by Association of Official Analytical Chemists (AOAC., 2005). At the end of the feeding trial 5 fish were randomly selected per treatment for proximate analysis. Diet performance was evaluated on experimental fish according to Olvera-Novoa *et al.* (1990).

Hematological profile: Blood samples were collected using heparinized plastic syringe (2 mL) fitted with 21 gauge hypodermic needle at day 0th and 84th. Blood samples were preserved in disodium salt of Ethylene Diamine Tetra-Acetic Acid (EDTA) bottles for analysis as described by Joshi *et al.* (2002).

Statistical analysis: Data representing mean values were subjected to one way analysis of variance at p>0.05 using SPSS (Statistical Package Computer Software, 1988 version, Chicago Illinois, USA). Duncan Multiple Range Test was used to compare differences between means.

RESULTS

The proximate composition of the diets is as presented in Table 1. Crude protein in experimental diets ranged from 39.98% in OSM to 41.83% in OSM+M, while the highest moisture content was recorded in PSM (12.00%) and least value of 8.32% recorded in NSM.

Results of the growth performance and nutrient utilization are presented in Table 2, which revealed that weight gain varied significantly (p<0.05) among treatments with the highest weight gain (22.77 g) recorded in PSM groups and the least (17.54 g) recorded in NSM groups. This same trend was observed in the final length of experimental fish with values ranging from 14.63 cm in NSM to 16.90 cm in PSM. The PER and SGR were significantly higher (p<0.05) in PSM, followed by OSM+M, while the least values were observed in NSM. Superior FCR was recorded in PSM, while the most inferior value was recorded in NSM. However, survival rate was statistically similar in all groups.

The initial and final proximate composition of the experimental fish shows that crude protein and fat contents were significantly higher in all experimental fish when compared with the initial value as presented in Table 3. However, ash content varied significantly (p<0.05) with treatments with values ranging from 9.80% in the initial through 12.69% in OSM groups to 13.64% in OSM+M groups.

The PCV values of all experimental fish decreased from 30.33% recorded as the initial value to 13.88, 17.33 and 20.66 in PSM, NSM and both OSM and OSM+M, respectively. Amongst treatments, the OSM+M groups had a significantly was recorded in OSM+M while NSM had the highest value $(3.92 \times 10^4 \text{ mm}^{-3})$. The MCV values ranged from $47.88 \text{ } \mu \text{m}^3$ in NSM to $85.14 \text{ } \mu \text{m}^3$ in OSM+M as shown in Table 4.

Table 4: Initial and pooled mean of Heamatological parameters of O. niloticus fed experimental diets (Mean ±SD)

Parameters	Initial	PSM	NSM	OSM	OSM+M
PCV (%)	30.33±0.57 ^a	13.88±1.99 ^d	17.33±1.52°	20.66±1.15 ^b	22.66±0.57b
Hb (g dL^{-1})	9.33 ± 0.98^{a}	5.53±0.01 ^d	5.37±0.02 ^d	6.63±0.05°	7.87 ± 0.08^{b}
RBC (cellsx10 ⁶ mm ⁻³)	2.43 ± 0.05^{d}	2.53±0.05°	2.40 ± 0.04^{d}	2.81±0.01ª	2.66±0.05 ^b
WBC (cellsx10 ⁴ mm ⁻³)	16.23 ± 0.15^{a}	3.30 ± 0.00^{d}	3.92±0.01 ^b	3.64±0.03°	3.12 ± 0.00^{e}
Platelet (109 L ⁻¹)	10.00 ± 0.00^a	5.16±0.28bc	4.47±0.13°	5.65±0.05 ^b	6.00±0.01 ^b
MCV (µm³)	12.33±0.05 ^e	65.99±0.01°	47.88±0.19 ^d	69.55±0.38 ^b	85.14±0.16 ^a
MCH (pg cell ⁻¹)	40.00 ± 0.00^a	21.69±0.05 ^e	22.03±0.05 ^d	23.03±0.05°	29.96±0.06b
MCHC (g dL^{-1})	33.00 ± 0.00^a	32.65±0.05 ^b	33.03 ± 0.05^{a}	32.64±0.03 ^b	33.00 ± 0.00^{a}
LYM (%)	65.00 ± 0.00^{c}	74.77 ± 0.19^a	64.32±0.00 ^d	60.00 ± 0.00^{e}	70.00 ± 0.00^{b}
NEUT (%)	29.00 ± 0.00^{c}	24.33±0.57 ^d	34.66±0.01 ^b	39.00 ± 0.10^{a}	28.66±0.20°
MONO (%)	4.00 ± 0.00^{a}	1.33±0.33 ^b	1.00±0.00 ^b	1.00±0.00 ^b	1.33±0.57 ^b

Mean with same superscript along the same row are not significantly different (p>0.05), PSM: Soybean meal inclusion, NSM: No soybean meal, OSM: Only soybean meal, OSM+M: Only soybean meal and methionine, HB: Haemoglobin, RBC: Red blood cells, WBC: While blood cell, MCV: Mean corpusculer volume and MCH: Mran corpuscular haemoglobin

Table 5: Plasma biochemistry of *O. niloticus* fed experimental diets (Mean±SD)

Parameters	Initial	PSM	NSM	OSM	OSM+M
Total protein (g dL ⁻¹)	4.01 ± 0.00 ^a	2.20±0.00e	2.36±0.04 ^d	2.52±0.03°	3.76±0.01 ^b
Albumin (g dL ⁻¹)	1.50 ± 0.01^{a}	0.76 ± 0.00^{e}	0.96±0.01°	0.90 ± 0.00^{d}	1.20±0.01 ^b
Globulin (g dL ⁻¹)	2.60 ± 0.00^{a}	1.43±0.01 ^c	1.44 ± 0.00^{c}	1.26±0.01 ^d	2.53±0.02b
ALT (U L ⁻¹)	29.20 ± 0.00^{d}	28.64±0.03e	30.00±0.01°	34.00±0.05 ^b	46.66±0.00°
AST (U L ⁻¹)	128.23 ± 1.06^a	47.66 ± 0.03^{d}	46.33 ± 0.00^{e}	54.00±0.01°	68.00±0.00 ^b
Glucose (mg dL^{-1})	50.10±0.05 ^e	51.33±0.01 ^d	51.66±0.00°	57.33±0.21 ^b	70.00 ± 0.08^{a}

Mean with same subscript along the same row are not significantly different (p>0.05), AST: Aspartate aminotrans ferase, ALT: Alanine aminotransferase, PSM: Soybean meal inclusion, NSM: No soybean meal, OSM: Only soybean meal and OSM+M: Only soybean meal and methionine

Table 6: Mean values of water quality parameters during experimental period

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Parameters	PSM	NSM	OSM	OSM+M
Temperature (°C)	28.0	28.2	28.3	28.0
рН	7.20	6.90	7.90	7.60
Dissolved oxygen (mg L^{-1})	3.90	3.80	4.10	4.80
Ammonia-nitrogen (mg L^{-1})	0.05	0.09	0.05	0.04
Nitrite-nitrogen (mg L ⁻¹)	0.01	0.02	0.01	0.01

PSM: Soybean meal inclusion, NSM: No soybean meal, OSM: Only soybean meal and OSM+M: Only soybean meal and methionine

All plasma biochemical parameters examined in this present study were significantly higher (p<0.05) in OSM+M groups when compared with other treatments as shown in Table 5. Total plasma protein ranged from 2.20 g dL $^{-1}$ in PSM to 3.76 g dL $^{-1}$ in OSM+M.

Result of water quality parameters measured during the study period is presented in Table 6. Temperature, pH and DO values ranged from 28.0-28.3 $^{\circ}$ C, 6.9-7.9 and 3.8-4.8 mg L⁻¹, respectively.

DISCUSSION

Growth and nutrient utilization were significantly high (p<0.05) in the PSM and OSM+M groups, in contrast to NSM and OSM groups. This indicates that partial replacement of fishmeal with soybean meal did not compromise growth. Similar result was reported with partial replacement of up to two third of fishmeal with soybean meal in the diet of tilapia

(Shiau et al., 1990, 1989). Similar results were obtained with partial soymeal inclusion diets by Al-Kenawy et al. (2008), Borgeson et al. (2006), Furuya et al. (2004), El-Saidy and Gaber (2003) and Shiau et al. (1990). However, total replacement caused a reduction in growth and feed utilization as exhibited by the OSM groups. Soybean is reported to contain approximately 30% carbohydrate of which oligosaccharides make up 10% and Non-starch Polysaccharides (NSP) make up the remaining 20% (Storebakken et al., 2000). The carbohydrates are not digestible by monogastric animals, with some components having antinutritional effects in fish (Chou et al., 2004). The low growth and nutrient utilization in fish fed diet with complete replacement of fishmeal with soybean meal could be attributed to an increase in the carbohydrate component of the diet. Also, Koumi et al. (2008) observed lower growth in O. niloticus when fishmeal was totally replaced by soybean meal. Davis et al. (2005) reported a decrease in growth response of juvenile red snapper, Lutjanus campechanus when soyabean meal was totally used as a replacement for fishmeal. Methionine supplement improved growth and nutrient utilization parameters in O. niloticus. This result is in agreement with Shiau et al. (1987) and Murai et al. (1986) who both reported improved growth when diets of tilapia were supplemented with crystalline methionine. In contrast, El-Saidy and Gaber (2002) reported that feeding blue tilapia and Nile tilapia, respectively with 100% SBM, firstly with or without methionine supplementation had no significant effect on growth parameters and feed utilization.

Meanwhile, the reduced growth rate observed in the NSM group could be due to the fact that *O. niloticus* has a preference for plant protein as it possesses morphological and physiological adaptations for diets high in fibre content.

Ayoola (2010) opined that the protein quality of the feed fed the fish determines whether the feed material accepted by the fish is balanced or not. Proximate analysis of the carcass of PSM group revealed high percentage dry matter and crude protein. The highest crude fat and ash was obtained in SBM+M group and this may be due to the availability of sufficient energy in the diet. This assertion is supported by Abdelghany (2003), who reported that diets containing soybean meal for *O. niloticus* increased body crude protein.

According to Ranzani-Paiva et al. (2000) haematological characteristics of most fish arestudied with the aim of establishing any deviation from the normal value as this may indicate a disturbance in the physiological process. The values obtained from this study forthe hematological parameters were within normal range (Abdelghany, 2003). This may have accounted for the good activity and healthy appearance of all experimental fish. Similarly, survival rate was excellent for all treatment, showing no significant variation. Higher erythrocyte count in the OSM+M groups is indicative of higher oxygen absorption and transportation capacity (Akintayo et al., 2008). The effect of treatments on specific activities of alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were significant. According to Hansen et al. (2007), ALT and AST are two major enzymes which are quantitatively important in transamination of amino acids in the liver and kidney.

The ranges of water quality parameters were within tolerable levels for warm water fish growth and survival (El-Sayed, 2006). The 100% survival recorded in this present study is comparable to the values 98.8-100% reported by Coyle *et al.* (2004) when fishmeal was substituted with a mixture of various plant protein sources for juvenile hybrid tilapia (*O. niloticus x O. aureus*).

CONCLUSION

Considering growth, nutrient utilization and the health status of *O. niloticus*, soybean meal can partially replace fishmeal up to 46.7% and also replace it totally when supplemented with methionine.

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