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Growth performance and haematological responses of Oreochromis niloticus (Linnaeus) Fingerlings fed Pigeon Pea [Cajanus cajan (L.) Millsp]

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ABSTRACT

Feeding trial was conducted to evaluate the growth performance of Oreochromis niloticus fingerlings fed diets containing Pigeon pea seed meal (PSM) as an alternative protein source to soybean meal. Five iso-nitrogenous (crude protein-30%) diets were formulated containing Pigeon pea seed meal at A(0%PSM), B(25%PSM), C(50%PSM), D(75%PSM) and E(100%PSM) control, were fed at 5% to triplicates groups of 15 fingerlings (average initial weight of 3.80+0.57 g) of *O. niloticus* for twelve (12) weeks. Growth performance and nutrient utilization parameters indicate that D(75%PSM), gave the highest weight gain (8.51+1.45 g) and the lowest weight gain in B(25%PSM) (5.17+0.28). Similarly, the highest specific growth rate (7.33+2.86) was recorded in C(50%PSM). Feed conversion ratio and survival rate were not significantly different (P>0.05) among the dietary treatments. The result from this study indicated that Pigeon seed meal (PSM) can replace soybean meal up to 75% in the diet of *O. niloticus* without compromising growth performance, nutrient utilization and haematological parameters.

Keywords: Pigeon pea, Soybean meal, Growth performance, *Oreochromis niloticus*

INTRODUCTION

The expansion in the aquaculture industry has put pressure in the increasing demand for fish feed which is a major bottleneck faced by farmers in developing countries like Nigeria (Dienye HE et al. 2018). Feed represents a major part of the operational costs. Fish meal which is the conventional source of animal protein in the fish diet is expensive and it has been valued for its balanced amino acids, vitamin contents, palatability and growth factors (Tacon AGJ 1993 , Jamabo NA 2017 and FAO 2016) Soybean meal is currently the most commonly used plant protein source in the fish feed industry, amounting to 50% in the diet of catfish (Solomon SG et al. 2016) has a high protein content of 38-42%, good amino acid balance and digestibility (Baker KM 2009) The demand to substitute soybean with readily available plant protein sources in the practical diet of *O. niloticus* and other fishes is due to the various uses to which it is put; as a dietary ingredient for human consumption and feed ingredients by other animal feed industries (Ndau LJ et al. 2015). Considerable efforts have been focused on legumes as alternatives to expensive feed ingredient in Nigeria (Hammed AM et al. 2013). Investigations on the feasibility of replacing SBM with plant protein alternatives in the diets of tilapia have been increasing and generally, a partial replacement of between 20%-30% is possible without decreasing growth. Some of these have included rubber seed meal (Deng JM et al. 2015), velvet bean meal (Aderolu AZ et al. 2009), faba bean meal (Azaza MS et al. 2009 & Ibrahim R et al. 2018), green algae ulva meal (Azaza MS et al. 2008), or pigeon pea meal (Obasa SO et al. 2003).

The increasing demand, price, competitions with human needs and its use in other animal feeds of soybean has emphasized the need for alternative protein sources in fish feeds. It has, therefore, become vital to search for an alternative that is not in direct competition with human and other animals. Pigeon pea (Cajanus cajan) seed is one of the tropical legume seeds that has been scarcely used in fish feed production despite its crude protein and energy profile. Like other legume seeds, its nutritive value is masked by the occurrence of anti-nutritional factors, example trypsin inhibitors haemagglutinin and saponin (Grimand P 1988 and Francis G et al. 2001).

Fish haematology is gaining increasing importance in fish culture because of its significance in monitoring the health status of fish (Hrubec TC et al. 2000, Bahmani M et al. 2001 and Bahmani M et al. 2005). Blood parameters are an important tool for monitoring both the nutritional and health status of fish (Hlophe SN et al. 2014). Haematological components of blood are also valuable in monitoring toxicity especially with feed constituents that affect the formation of blood (Dienye HE et al. 2014). RBC, Hb and HCT decreased significantly in C. gariepinus fed high Moringa meal levels (Hlophe SN et al. 2014). This decrease further indicates nutritional stress (Hlophe SN et al. 2014). This study aims to evaluate the effect of replacing SBM with Pigeon pea seed meal (PSM) on growth, nutrient utilization and haematology of *O. niloticus*.

MATERIALS AND METHODS

Experimental Procedure

The experiment was carried out at the aquaculture unit of the University of Port Harcourt demonstration farm, in Port Harcourt, Rivers State, Nigeria. A total of 225 fingerlings of Oreochromis niloticus with an average weight of 3.80 g were acclimatized to experimental conditions for seven days. After acclimatisation, fifteen randomly selected fish were stocked into each of 15 aquarium tanks each of which had a size of 1.8 x 1.3 x 0.36 m3. Aeration of aquaria water was done using air pumps.

Experimental Design

Experimental fishes weighing 3.80+0.57 g were stocked in fifteen tanks each containing 15 fish. The fingerlings were randomly grouped into five treatments of fifteen fish per tank. The treatments were allocated as A(0%PSM), B(25%PSM), C(50%PSM), D(75%PSM) and E(100%PSM) control respectively. Each treatment was in triplicate. The fingerlings were fed at 5% of their body weight with the experimental diets for 12 weeks.

Feed Formulation and Diet Preparation

Feed ingredients used in this study were obtained from a local commercial supplier in Port Harcourt, Rivers State, Nigeria. The diets contained Fish meal, soybean meal, yellow maize meal, wheat brain and Pigeon pea seed meal (C. cajan), bone meal, vegetable oil, salt, vitamin premix, methionine and lysine. All the dietary ingredients were separately processed and milled to fine particle size. The feed ingredients were then weighed out to replace soybean meal at various inclusion levels. Five dry diets were formulated based on the varying inclusion levels of PSM, A(0%PSM), B(25%PSM), C(50%PSM), D(75%PSM) and E(100%PSM) following Pearson Square Method as describe by (Gohl B 1981). The ingredients were mixed until uniformly blended. Water was added slowly to the mixture with continuous stirring to form dough. The dough was pelleted with a hand pelletiser. Ingredients and composition of fish diet are shown in Table 1.

Ingredients	**A** $_{0\%PSM}$	**B** $_{25\%PSM}$	**C** $_{50\%PSM}$	**D** $_{75\%PSM}$	**E** $_{100\%PSM}$
PSM	-	11.45	22.9	34.35	45.8
SBM	45.8	34.35	22.9	11.45	-
Fishmeal	26.61	26.61	26.61	26.61	26.61
Yellow maize	18.51	18.51	18.51	18.51	18.51
Bone meal	1	1	1	1	1
Vitamin premix	1	1	1	1	1
Lysine	1	1	1	1	1
Methionine	1	1	1	1	1
Vitamin C	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5
Starch (binder)	0.5	0.5	0.5	0.5	0.5
Vegetable oil	3	3	3	3	3
Total	100	100	100	100	100

Table 1: Composition of experimental diet

Water Quality Monitoring

Water quality was measured twice a week at 7:00–8:00 before feeding. Temperature was recorded with a digital thermometer; dissolved oxygen was determined by Winkler's method and pH was determined with Hannah E251 pH meter and ionized ammonia; was measured using an ammonia assay kit.

Determination of Growth Performance

The evaluation of experimental diets for growth performance was carried out using growth indices such as Weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), feed intake (FI), protein efficiency ratio (PER) and survival as described by (Fashina-Bombata HA et al. 2010). All these parameters were measured for all the treatments and their replicates. Mortalities were recorded as they occurred.

*Specification: each kg contains: Vitamin A, 4,000,000 IU; Vitamin B, 800,000 IU; Vitamin E, 16,000 mg, Vitamin K3, 800 mg; Vitamin B1, 600 mg; Vitamin B2, 2000 mg; Vitamin B6, 1600 mg, Vitamin B12, 8 mg; Niacin,16,000 mg; Caplan, 4000 mg; Folic Acid, 400 mg; Biotin, 40 mg; Antioxidant 40,000 mg; Chlorine chloride, 120,000 mg; Manganese, 32,000 mg; Iron 16,000 mg; Zinc, 24,000 mg; Copper 32,000 mg; Iodine 320 mg; Cobalt, 120 mg; Selenium, 800 mg manufactured by DSM Nutritional products Europe Limited, Basle, Switzerland.

Proximate Analysis of Experimental Diets

Proximate analysis of the experimental diets was conducted according to the standard protocols of (AOAC 1989.2005). Moisture content was determined by drying samples in an oven at 70°C for 48 hours. Crude protein was determined according to Kjeldah method. Samples were digested in concentrated sulphuric acid by a digestor, distillated and titrated to obtained nitrogen. Crude protein was obtained by multiplying nitrogen content with a conversion factor of 6.25. Crude fibre was determined by digesting a sample with weak base preceded by a weak acid. Ankom 220 Fiber analyzer was used to determine the crude fibre. Crude lipid was determined after soxhlet extraction of dried samples with 1.25% H2SO4 and 1.25% NaOH Ash content was determined by calculation method. Samples which were previously oven-dried were put in crucible and heat in a muffle furnace at 550°C for 3 hours and then cooled before weighing

Haematological Parameters

At the end of the rearing trial, 8 fish were randomly removed from each diet treatment and blood was collected from the fish. Blood samples of about 2 milliliters were collected via the caudal peduncle puncture (AQUALEX 2004) with the aid of a 2 ml thermodynamic plastic syringe and needle, and the blood was dispensed into ethylene diamine tetra-acetic acid (EDTA) anticoagulant bottle for haematological studies. Haematology was done using Sysmex Hematological analyser, Coagulation Systems. The haematological parameters obtained include packed cell volume (PCV) or Haematocrit (HCT), Haemoglobin (Hb) concentration and red blood cell (RBC). The white blood cell (WBC) and differential count (monocyte, lymphocytes, neutrophils and eosinophil) were determined as described by (Dacie JV et al. 2011).

Statistical Analysis

Results were expressed as the mean +SE. Difference between groups was determined using one-way analysis of variance (ANOVA) with the statistical software package SPSS 16.0. Multiple range test was carried out to determine differences between treatment means (significance level, p<0.05).

RESULT

The proximate composition of the experimental diets is shown in Table 2. There was a significant difference in all the parameters measured apart from the fat content.

Parameters	$A_{0\%PSM}$	$B_{25\%PSM}$	$C_{50\%PSM}$	$D_{75\%PSM}$	$E_{100\%PSM}$
Moisture	$11.01 + 0.09^{ab}$	10.98 ± 0.54^{ab}	9.92 ± 0.15^{bc}	11.29 ± 0.51^{a}	9.48 ± 0.00^{c}
Crude Protein	32.41 ± 0.00^{a}	25.58 ± 0.43^{c}	26.39 ± 0.43^{c}	28.28 ± 0.44^{b}	28.20 ± 0.00^{b}
Crude Fat	5.67 ± 1.00^{a}	6.09 ± 0.91^{a}	6.72 ± 0.49^{a}	7.20 ± 0.34^{a}	6.39 ± 0.48^{a}
Crude Fibre	7.03 ± 0.00^{b}	7.56 ± 0.00^{b}	6.92 ± 0.65^{b}	10.26 ± 0.00^{a}	7.59 ± 0.00^{b}
Ash	11.86 ± 0.13^{a}	11.30 ± 0.07^{abc}	10.61 ± 0.31^{bc}	11.35 ± 0.15^{ab}	10.55 ± 0.29^{a}
CHO	32.02 ± 1.22^{b}	38.49 ± 0.87^{a}	40.09 ± 1.09^{a}	31.63 ± 0.57^{b}	37.98 ± 0.77^{a}

Table 2: Proximate composition of experimental diets

*Superscripts of the same alphabet are not significantly different (P>0.05)
**Superscripts of different alphabets are significantly different (P<0.05)

Physico-chemical Parameters of Experimental Waters

The summary of the Physico-chemical parameters of the experimental waters during the period of the study is shown in Table 3. The result reveals that values of all the parameters significantly differ except temperature.

Parameters	$A_{0\%PSM}$	$B_{25\%PSM}$	$C_{50\%PSM}$	$D_{75\%PSM}$	$E_{100\%PSM}$
Temperature(˚C)	25.23 ± 0.15[a]	25.17 ± 0.07[a]	25.21 ± 0.01[a]	25.25 ± 0.05[a]	25.26 ± 0.08[a]
pH	6.58 ± 0.12[a]	6.23 ± 0.06[c]	6.13 ± 0.02[a]	6.20 ± 0.01[a]	6.39 ± 0.01[a]
DO (mg/l)	3.83 ± 0.13[c]	3.67 ± 0.04[b]	3.56 ± 0.10[b]	4.74 ± 0.03[c]	5.08 ± 0.03[a]
Ammonia (mg/l)	0.55 ± 0.05[b]	0.39 ± 0.04[c]	0.60 ± 0.06[b]	1.15 ± 0.01[a]	0.69 ± 0.03[b]

Table 3: Physio-chemical Parameters of Water

*Superscripts of the same alphabet are not significantly different (P<0.05)
**Superscripts of different alphabets are significantly different (P<0.05)

Growth Performance

The result of growth performance is shown in Table 4. The best feed intake and weight gain were observed in treatment D75%PSM. Feed conversion ratio was highly efficient in treatment D75%PSM. Survival rate was high in treatment B25%PSM. Protein intake was lowest in treatment A0%PSM and B25%PSM and the highest value was obtained in treatment D75%PSM.

Parameters	$A_{0\%PSM}$	$B_{25\%PSM}$	$C_{50\%PSM}$	$D_{75\%PSM}$	$E_{100\%PSM}$
No. of fish stocked	15	15	15	15	15
Duration of experiment	128	128	128	128	128
Initial mean weight (g)	3.8 ± 0.57[a]	3.75 ± 0.78[a]	3.72 ± 0.27[a]	3.68 ± 0.21[a]	3.74 ± 0.40[a]
Final mean weight (g)	6.20 ± 0.28[a]	5.17 ± 0.28[b]	6.37 ± 1.45[ab]	8.51 ± 1.45[a]	6.14 ± 0.21[a]
Mean weight gain (g)	2.35 ± 0.63[a]	1.83 ± 0.62[a]	3.10 ± 1.29[a]	4.83 ± 1.63[a]	2.40 ± 0.49[a]
Specific growth rates (SGR%)	6.20 ± 1.87[a]	5.51 ± 2.07[a]	7.33 ± 2.86[a]	7.17 ± 2.55[a]	6.51 ± 0.86[a]
Survival rate	9.33 ± 2.40[a]	12.33 ± 0.33[a]	10.67 ± 1.86[a]	7.67 ± 1.20[a]	10.33 ± 1.45[a]
Feed Intake	2.25 ± 0.12[ab]	1.78 ± 0.12[b]	2.15 ± 0.26[ab]	2.62 ± 0.09[a]	2.07 ± 0.13[b]
Feed conversion ratio (FCR)	1.17 ± 0.39[a]	1.43 ± 0.68[a]	1.31 ± 0.73[a]	0.92 ± 0.27[a]	0.95 ± 0.24[a]
Protein Efficiency ratio (PER)	0.07 ± 0.02[a]	0.07 ± 0.02[a]	0.11 ± 0.05[a]	0.12 ± 0.05[a]	0.09 ± 0.01[a]

Table 4: Growth performance of *O. niloticus* fed with experimental diet

*Superscripts of the same alphabet are not significantly different (P<0.05)
**Superscripts of different alphabets are significantly different (P<0.05)

Haematological Parameters

After 12 weeks of rearing trials, statistical analysis of data showed that there were differences on the PCV, Hb, RBC and WBC count among the treatments (p<0.05). Highest PVC, Hb and RBC values were obtained in treatment E100%PSM. Platelets, eosinophil and monocytes values were similar (P>0.05) across the treatment (Table 5).

Parameters	$A_{0\%PSM}$	$B_{25\%PSM}$	$C_{50\%PSM}$	$D_{75\%PSM}$	$E_{100\%PSM}$
PVC(%)	9.33 ± 0.67[b]	10.00 ± 1.16[b]	10.67 ± 0.67[a]	12.00 ± 1.16[a]	13.33 ± 0.67[a]
Hb(g/dl)	3.10 ± 0.20[b]	3.33 ± 0.38[b]	3.53 ± 0.23[ab]	4.00 ± 0.40[a]	4.47 ± 0.23[a]
RBC(×106 m-1)	2.10 ± 0.53[b]	2.33 ± 0.26[ab]	2.40 ± 0.15[ab]	2.67 ± 0.24[ab]	2.87 ± 0.09[a]
WBC(×103 m-1)	5.10 ± 0.26[a]	4.83 ± 0.09[a]	4.50 ± 0.17[b]	4.16 ± 0.12[b]	3.76 ± 0.15[a]
Platelets(x109/l)	68.00 ± 3.79[b]	66.67 ± 2.73[a]	63.00 ± 3.79[a]	63.33 ± 4.91[a]	58.33 ± 5.24[b]
Neutrophils(%)	18.67 ± 2.40a	16.67 ± 3.28[b]	24.00 ± 2.08[a]	24.67 ± 3.53[a]	24.33 ± 1.45[b]
Lymphocytes(%)	68.33 ± 3.76[a]	62.67 ± 4.33[a]	63.67 ± 2.96[b]	59.33 ± 4.33[b]	62.33 ± 1.45[b]

Eosinophils(%)	4.00 ± 0.58[b]	3.00 ± 0.58[b]	3.33 ± 0.88[a]	5.67 ± 1.20[a]	4.33 ± 1.33[b]
Monocytes(%)	9.00 ± 2.08a	7.67 ± 1.45[a]	9.00 ± 2.08[b]	10.33 ± 0.33[a]	9.00 ± 1.53[b]
Protein Efficiency ratio (PER)	0.07 ± 0.02[a]	0.07 ± 0.02[a]	0.11 ± 0.05[a]	0.12 ± 0.05[a]	0.09 ± 0.01[a]

Table 5: Haematological parameters of O. niloticus fed with the experimental diet

Data are represented as mean ± SE. Superscripts of different alphabets are significantly different (P<0.05)

DISCUSSION

Fish nutritionist has made attempts to substitute soybean meal partially or totally in the feed formulation. There are numerous agriculture products and forage crops produced in Nigeria which are utilized as alternative protein sources for tilapia culture. Growth in fish depends on the nutritive quality of feeds, especially its crude protein. The proximate composition of the experimental diets for crude protein was similar (35%) for all the treatment diets. The crude protein value recorded in the experimental diets were similar to what (Ndau LJ et al. 2015) reported for *O. niloticus*, while crude fiber was appreciable within the acceptable range requirement for fish. The high ash content of the experimental treatment is the indication of high minerals inherent in the treatment diets. Water quality parameters recorded in all the treatment tanks were within the acceptable ranges for tilapia fish growth and health (Beveridge MCM et al. 2000). The experiment on the evaluation of *O. niloticus* fed with different inclusion levels of PSM using same ingredients in varied proportions has shown no significant difference among the diets (p>0.05) which means that it had no adverse effect on the growth performance this proves that PSM could be included at different levels or could be used to replace soybean meal without affecting the growth of *O. niloticus*. This is supported by the study conducted by (Hammed AM et al. 2013), where they reported that PSM could replace soybean meal in C. gariepinus diet without having any adverse effect on the growth of the fish. (Obasa SO et al. 2003). Observed that PSM could replace soybean meal in the diet of *O. niloticus. This is also in agreement with the works of (Mukhopadhyay N et al. 1999 and Mukhopadhyay N et al. 2001*) who observed that plant-derived proteins such as linseed meal and sesame seed meal could replace fish meal at levels as high as 50% when fortified with amino acids. Reported good growth rates for C. gariepinus when PSM was partially replaced at (25%) or total replacement (100%) for soybean meal in their diet. Studies have shown that the higher the feed conversion ratio, the less desirable the diet is. As animals consume more feed to produce a unit of weight gain. Feed conversion ratio is usually influenced by various factors like the quality of feed given, pond water quality and feeding management. The best FCR recorded in treatment D75%PSM implies that the fish utilized the supplied feed with the highest efficiency.

Haematological parameters are valuable indicators for monitoring fish health, nutritional status and environmental conditions affecting fish (Hoseinifar SH et al. 2011, Soyinka OO et al. 2015 and Rufchaie R et al. 2014). The most common blood characteristics constantly affected by diet are the RBC and Hb. Significant differences in PVC, Hb, RBC and WBC content were found in fish diets with different PSM levels. The increase observed in the values of packed cell volume when the fish was fed experimental diets could be due to anaemia resulting from shrunken red blood cells. The values recorded in this study for PVC recorded are in agreement with (Akinrotimi OA et al. 2011) they reported a reference range of African catfish PVC (32.64-46.74) and Hb (10.02-18.64). The values of RBC, Hb and WBC are within the range reported for normal, healthy juvenile of *O. niloticus* (Ndau LJ et al. 2015). Our results show that PSM had no remarkable effects on all the haematological parameters. Moreover, studies will be done to attain some data between the relationship of haematological indices and fish health.

CONCLUSION

The present study indicates that Pigeon pea seeds are valuable feed ingredient and can be used to replace soybean meal in the diet of *O. niloticus*, because of its acceptability and utilization by the fish as well as its effect on haematological parameters as it has no adverse effect on the blood profile.

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