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Growth and economic performance of Nile Tilapia, *Oreochromis niloticus* (L.) fingerlings fed diets containing graded levels of sclerotium

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Abstract. This study was conducted to evaluate the effects of inclusion of sclerotium as a substitute for soybean meal in the diet of *Oreochromis niloticus* using growth performance, economy of production, haematological and biochemical parameters as indices. Four experimental diets comprising of control (CTR) without sclerotium and other three diets SC1, SC2 and SC3 containing 7.5, 10.0 and 12.5 % sclerotium, respectively. The experimental diets were fed to triplicate groups of fish fingerlings (0.81± 0.00g average body weight) for 70 days. The inclusion of sclerotium at 7.5% level recorded the best values for mean weight gain (MWG), specific growth rate (SGR) and feed conversion ratio (FCR). There was no significant difference ($p>0.05$) in the incidence cost (IC) and profit index (PI) among the diets however, the best results were achieved at 10% (SC2) inclusion level. The parked cell volume (PCV), haemoglobin (Hb), neutrophil and lymphocyte showed significant difference ($p<0.05$) between CTR and other three diets while there was no significant difference ($p>0.05$) in eosinophil among the four diets. Biochemical parameters recorded significant differences ($p<0.05$) among the four diets. Hence, due to the nutritional and therapeutic characteristics of sclerotium, its inclusion in the diet up to 12.5% could be tolerated by *O. niloticus*.

Key Words: sclerotium utilization, haematological, biochemical, *Oreochromis niloticus.*

Introduction. The need to substitute fishmeal in animal feed has necessitated the use of some protein rich plant feedstuffs. Legume seeds have been highly favourable because of their rich protein composition, energy and mineral content and widespread distribution in the tropics (Ogunji et al 2005). However, only few of these plant proteins have been utilized and investigated (Ogunji & Wirth 2001). The presence of anti-nutritional factors in these feedstuffs has limited their widespread usage and direct incorporation into animal feeds (Ogunji et al 2005). The evaluation of alternative protein sources to fishmeal is therefore a research priority. Single Cell Protein (SCP) including micro algae, bacteria and yeast are alternative protein sources that are used as feed ingredients for fish (Salnur et al 2009). SCP consists of different nutrient elements such as proteins, B-vitamins, pigments, complex carbohydrates and glucan (Tacon 1994). Compared to fishmeal, the majority of the SCP are either deficient in one or more amino acids or they suffer from an amino acid imbalance (Kiessling & Askbrandt 1993). The supplementation of yeast-based diets with the deficient amino acids was shown to have beneficial effects on fish growth (Murray & Marchant 1986). In most monogastric animals, an excess of dietary nucleic acids supply is toxic, as the capacity of excretion of the uric acid formed is limited, leading to deposits of uric acid in the body and possible disorder of metabolism (Tuse 1984). However, no such effect was found in fish due to their very active liver uricase (Rumsey et al 1991). Tilapia is well suited to warm and tropical temperatures, resistant to diseases and tolerant to stress. They respond favourably to new technologies in breeding, genetics, feed production and rearing techniques. Tilapia will grow on a wide variety of nutrient sources from pond algae and bacteria to high quality feedstuffs such as grains, oil seeds and fishmeal (Stickney 2000). Yeasts have been the most used within aqua feeds (Tacon 1994) because *Saccharomyces cerevisiae* is believed to have immuno-stimulatory properties, due to its complex carbohydrate components and nucleic acid content (Anderson et al 1995). The use of sclerotium of higher fungus (*Pleurotus tuber-regium*) in the diet of Nile tilapia (*Oreochromis niloticus*) has been little investigated. This study therefore evaluates the substitution effects of graded level of sclerotium in the diet of Nile tilapia fingerlings using growth performance, nutrient utilization, economic performance, haematological and biochemical parameters as indices.

Materials and Methods. The experiment was carried out between October and December 2011 at the Fish Nutrition Unit, Department of Marine Sciences, University of Lagos, Akoka, Nigeria.

Collection and processing of sclerotium. Sclerotium is a compact mass of hardened mycelium stored with reserve food material that, in some higher fungi such as ergot, becomes detached and remains dormant until a favourable opportunity for growth occurs (Calvo 2008). *P. tuber-regium* was obtained from a local market, Oyingbo market in Lagos Nigeria. The sclerotium was peeled and cut into small bits and blended with kitchen blender to achieve smooth powdery product, which was mixed with other feed ingredients. The proximate compositions of sclerotium and soybean meals were carried out at the Department of Animal Science, University of Ibadan, Nigeria according to the Association of Analytical Chemists Method (AOAC 2004) (Table 1).

Table 1

Proximate composition of sclerotium and soybean

Composition	*Sclerotium*	*Soybean*
Moisture	7.35	7.92
Protein	19.45	42.00
Ether extract	6.30	16.90
Ash	5.04	6.00
Fibre	18.02	5.34
Nitrogen Free Extract	43.84	21.84

Experimental animals and feeding regimes. A total of one hundred and twenty (120) fingerlings of Nile tilapia, *O. niloticus* (0.81±0.00 g average body weight) were purchased from Nouvos Farm, Agric area, Iyana-Oba, Lagos, Nigeria. Water was changed every two (2) days with de-chlorinated water from a borehole to maintain good water quality. The dissolved oxygen ranged from 4.5 to 6.0 mg L^{-1} while pH ranged from 6.5 to 7.0 during the experimental period.

Table 2

Gross composition of experimental diets containing graded levels of sclerotium as a replacement for soybean meal

Ingredients (%)	*Experimental diets*			
	CTR (g)	*SC1 (g)*	*SC2 (g)*	*SC3 (g)*
Fish meal (72%)	25.00	25.00	25.00	25.00
Groundnut cake	6.00	6.00	6.00	6.00
Soybean meal	25.00	17.50	15.00	12.50
Maize	36.00	36.00	36.00	36.00
Wheat offal	3.00	3.00	3.00	3.00
Sclerotium	-	7.50	10.00	12.50
Dicalcium sulphate	2.00	2.00	2.00	2.00
Soy oil	2.00	2.00	2.00	2.00
Vit. Premix	0.25	0.25	0.25	0.25
Lysine	0.30	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.20
Salt	0.25	0.25	0.25	0.25
Calculated nutrients value of the feed				
Crude protein (%)	35.31	35.09	35.05	35.02
Lipids (%)	5.90	5.87	5.86	5.86
Dietary fibre (%)	3.35	3.45	3.48	3.51
Energy-Protein ratio (Kcal/g)	10.14	10.17	10.18	10.18

Based on the nutrient composition of the feedstuffs, four iso-calorific and iso-nitrogenous experimental diets were formulated comprising of control (CTR) diet without sclerotium and three test diets; SC1, SC2, and SC3 containing 7.5, 10.0 and 12.5% sclerotium, respectively, as a replacement for soybean (Table 2). The diets were fed to triplicate groups of 10 fish at 5% body weight/day, twice a day (09:00 and 16:00 h) for 70 days. Fish were weighed collectively at 14 day intervals and their average weights recorded and the daily rations were adjusted accordingly.

Growth performance and nutrient utilization*.* Growth was estimated in terms of mean weight gain (MWG, g), specific growth rate (SGR, % d^{-1}), feed intake (FI, g) and feed conversion ratio (FCR) according to Morais et al (2001):

MWG = Mean Final body Weight (g) - Mean Initial body Weight (g)

SGR = [(ln final body weight - ln initial body weight)/rearing duration in days] x 100

FCR = Dry weight of feed fed (g)/Fish weight gain (g)

FI= Feed intake during experimental period (g)/No of days

Economy of production*.* The economic evaluation of the diets was calculated in terms of Incidence of cost (IC, ₦ g^{-1}) and Profit index (PI) from the method of New (1989). Cost was based on the current prices of feed ingredients in the experimental locality (Nigeria) at the time of the experiment.

IC = Cost of feed (₦)/Mean weight gain (g)

PI = Value of fish (₦)/Cost of Feed (₦)

Haematological and biochemical analyses*.* The parked cell volume (PVC), haemoglobin (Hb), neutrophil, lymphocyte and eosinophil of the fish blood were analysed using the method of Joshi et al (2002). The aspartic acid transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were measured according to the method described by Metwally (2009).

Statistical analysis*.* The experimental design was a complete randomized design and data were subjected to one way analysis of variance (ANOVA), Duncan's multiple range test was used to evaluate the mean difference among diets at 5% significant level. Equal variances were assumed.

Results. The growth and economic performance of *O. niloticus* fed experimental diets containing graded levels of sclerotium as a replacement for soybean meal is presented in Table 3. There was no significant difference ($p > 0.05$) in the mean weight gain (MWG) as well as the specific growth rate (SGR). The highest MWG and SGR were recorded in SC1 and the least in CTR. Although SC1 recorded the best performance for FCR, the feed intake (FI) and feed conversion ratio (FCR) recorded significant differences ($p < 0.05$) across diets. The incidence of cost (IC) and profit index (PI) did not show any significant difference ($p > 0.05$) among the four diets, however the least IC with highest PI was recorded at 10% (SC2) inclusion level while the least PI was recorded at 12.5% (SC3) inclusion level.

The results of the haematological parameters are shown in Table 4. Though there was no significant difference ($p > 0.05$) in the values of PCV and Hb for diets SC1, SC2 and SC3, nevertheless CTR had the highest values compared to these diets. With the exception of eosinophil, CTR showed significant difference ($p < 0.05$) in the volume of neutrophil and lymphocyte compared to other diets.

The results of the biochemical parameters are presented in Table 5. There were significant differences ($p < 0.05$) across the test diets for the three enzymes (AST, ALT and ALP) analyzed. In addition, significant drop was recorded in the values of the three enzymes with the increase in the inclusion levels of the test ingredient.

Table 3
Growth and economic performance of *O. niloticus* fed graded levels of sclerotium as a replacement for soybean meal

Parameters	*Experimental diets*			
	CTR	*SC1*	*SC2*	*SC3*
Mean Initial Weight (g fish^{-1})	0.81±0.00	0.81±0.00	0.81±0.00	0.81±0.00
Mean Final Weight (g fish^{-1})	4.08±0.01	4.54±0.18	4.52±0.14	4.29±0.21
Mean Weight Gain (g fish^{-1})	3.27±0.01	3.73±0.18	3.70±0.14	3.48±0.21
Specific Growth Rate (% d^{-1})	2.30±0.01	2.45±0.05	2.37±0.07	2.37±0.07
Feed Intake (g)	4.91^{b}±0.06	4.20^{a}±0.06	4.77^{b}±0.15	5.25^{c}±0.06
Feed Conversion Ratio	1.50^{b}±0.02	1.13^{a}±0.06	1.37^{b}±0.06	1.42^{b}±0.07
Incidence of Cost (₦)	0.23±0.00	0.17±0.01	0.16±0.05	0.21±0.01
Profit Index (₦)	19.63±0.26	22.82±0.33	36.16±0.28	18.60±0.23

Figures in each row with different superscript are significantly different (P < 0.05) from each other.

Table 4
Haematological indices of *O. niloticus* fed graded levels of sclerotium as a replacement for soybean meal

Parameters	*Experimental diets*			
	CTR	*SC1*	*SC2*	*SC3*
PCV (%)	41.00^{a}	29.00^{b}	30.67^{b}	29.00^{b}
Hb	14.37^{a}	9.53^{b}	9.83^{b}	9.67^{b}
Neutrophil (x10^{3}.µL^{-1})	51.00^{b}	52.00^{bc}	52.87^{c}	53.00^{a}
Lymphocyte (x10^{3}.µL^{-1})	48.00^{b}	50.07^{a}	50.33^{a}	50.20^{a}
Eosinophil (x10^{3}.µL^{-1})	0.00	0.33	0.67	0.50

Figures in each row with different superscript are significantly different (P < 0.05) from each other.

Table 5
Biochemical Parameters of *O. niloticus* fed graded levels of sclerotium as a replacement for soybean meal

Parameters	*Experimental diets*			
	CTR	*SC1*	*SC2*	*SC3*
AST(IU/L)	92.00^{c}	89.67^{d}	83.07^{a}	81.33^{b}
ALT(IU/L)	34.33^{d}	29.00^{a}	26.67^{b}	25.00^{c}
ALP(IU/L)	56.62^{d}	53.16^{c}	50.04^{b}	29.35^{a}

Figures in each row with different superscript are significantly different (P < 0.05) from each other.

Discussion. *P. tuber-regium* sclerotium may not have been documented in the diet of fish, but its consumption by humans has been reported by Fasidi & Olorunmaiye (1994). In traditional medical practice in Nigeria, it is used in preparation of drugs for headache, stomach ailments, cold and fever, asthma, smallpox and high blood pressure (Okhuoya & Okogbo 1991; Fasidi & Olorunmaiye 1994; Aloba 2003). Its inclusion in the Nile tilapia feed showed a better result in the test diets than the control diet in the present study. The obtained results clearly showed that, the replacement of up to 12.5% soybean meal (SBM) by sclerotium allowed for better growth (MWG and SGR) as against the control diet, similar result was observed by Metailler & Huelvan (1993) who tested the inclusion of 10, 20 and 30% of lactic yeast, baker's yeast and brewer's yeast in the diets of sea bass (*Dicentrarchus labrax*). The result was further corroborated by Oliva-Teles & Goncalves (2001) who checked the inclusion of up to 30% dietary protein from brewer's yeast in the diet of sea bass. Similar trend was also observed for the economic parameters (IC and PI), and feed utilization parameters (FCR). These results agreed with

Shalaby (2004), who found that Nile tilapia fed diet containing 2% fenugreek seed meal (FKSM) had significant higher body weight, MWG and SGR. Mostafa et al (2009) reported the same result with 1% FKSM in Nile tilapia feed. The improvement in the final body weight, MWG and SGR may be due to antibacterial effect of flavonoids in sclerotium (Ikewuchi & Ikewuchi 2008). Similar observations were reported of flavonoids in FKSM (Bhatti et al 1996) and in marjoram leave when fed to Nile tilapia fingerlings (Abd El-Maksoud et al 1999).

Haematological parameters have been recognized as valuable tools for the monitoring of fish health (Schutt et al 1997). They help fish biologists to interpret physiological responses to stress imposed by the food composition (Bouziane et al 1994; Secombes 1994). However, these parameters decrease in the presence of anti-nutritional factors (Osuigwe et al 2007). According to Ikewuchi & Ikewuchi (2008) sclerotium contains anti-nutritional factors (phytates and tannins). Phytic acid binds calcium, iron, zinc and other minerals, thereby reducing their availability in the body (FAO 1990) while tannins reduce blood cholesterol (Basu et al 2007). Hence the reduction in the values of PCV and Hb observed in this study with increased level of sclerotium could be attributed to the presence of these anti-nutritional factors. This study corroborates the work of earlier researchers who reported a decrease in haemocrit and haemoglobin content with increase level of ingredients (Blom et al 2001; Dabrowski et al 2001; Richard et al 2003). Furthermore, since the values of PCV and Hb recorded in this study were significantly below that of the control, it could be suggested that the diets tested had major physiological stress on the health status of the fish (Aderolu et al 2009). Consequently, the elevated values recorded in lymphocyte and eosinophil were responses to the stress caused by the anti-nutritional factors in sclerotium. Similarly, Martins et al (2008) reported the increase in number of neutrophils due to stress in Nile tilapia experimentally infected with *Enterococcus* sp. The increment recorded in the neutrophil value with increase in the inclusion level of test ingredient in this study may be due to the stress induced by anti-nutrients present in the diets.

Attention has been focused on the changes in AST, ALT and ALP activities, which promote gluconeogenesis from amino acid, as well as on the changes in aminotransferase activities in the liver (Hilmy et al 1981). Results of the present study showed reduction in AST, ALT and ALP, with the increased inclusion levels of sclerotium, this was similar to the report of El-Shater et al (1997) and Augusti et al (2001), who found that the lipid parameters and enzymes (AST, ALT, and ALP) activities in serum of rats decreased significantly when they were fed on a diet containing 5% *Allium sativum*. Similar result was reported by Shalaby et al (2006), who reported a decrease in ALT of Nile tilapia serum after being fed with a diet containing garlic (*A. sativum*) and chloramphenicol. These results can be attributed to the presence of anti-nutrients in sclerotium.

Conclusions. The findings of this study showed that graded levels of the test ingredient could be used as replacement for soya bean meal, without any adverse effect on growth and well-being of the fish. Hence, due to the nutritional and therapeutic characteristics of sclerotium, its inclusion in the fish diet up to 12.5% is recommended for *O. niloticus*.

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