EFFECTS OF DIET WITH BANANA LEAF MEAL (*MUSA ACUMINATA*) ON THE FECUNDITY, EGG SIZE AND GONADOSOMATIC INDEX OF THE NILE TILAPIA (*OREOCHROMISNILOTICUS*)

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Abstract

This study was conducted to evaluate the effects of diet formulated with Bananna leaf meal (Musa sp) on the fecundity, egg size and gonadosomatic index of the Nile Tilapia (Oreochromisniloticus). This feeding trial was conducted for one hundred and twenty (120) days using a triplicate setup of six (6) tarpaulin tanks measuring (100 x 100 x 100 cm^3) labeled A_l, A_2, A_3, B_l, B_2 and B_3. Two isonitrogenous diets containing a crude protein level of 35 % (Diet A and Diet B) were used in feeding the experimental fishes. Diet A was the control diet and Diet B was formulated with the inclusion of Banana leaf meal (BLM) in addition to dietary feed ingredients. A total of one hundred and fifty (150) fingerlings of O. niloticuswith a mean body weight of 2.84 ± 0.11 g and mean total length 3.96 ± 0.12 cm were purchased from a private fish farm in Uyo, AkwaIbom State and used for the study (25 fingerlings per tank). Results obtained showed that there was no significant difference ($p>0.05$) in the fecundity, egg size and gonadosomatic index of O. niloticus fed the two experimental diets which indicates that Banana leaf meal (BLM) did not negatively affect the fish reproductive indices. Physicochemical parameters including pH, water temperature (ºC), dissolved oxygen (mg/L) and ammonia level (mg/L) were maintained within the optimum range recommended for optimal growth and health of freshwater fishes. However, the inclusion of Banana leaf meal (BLM) in the diet of O. niloticus resulted in a reduction in the quantity of other conventional feed ingredients such as soybean meal (SBM), fish meal (FM), groundnut meal (GNM) and corn meal (CM). For a cost effective production of O. niloticus, the inclusion of Banana leaf meal (BLM) in the diet of O. niloticus is therefore recommended.

Keywords: *Plant-based protein source; Number of eggs; Egg diameter; Ovary development; Testis development*

Introduction

Banana is a fruit crop which generates forage material during production in quantity that can be utilized in feeding livestock including fishes in fresh, dried or ensiled form (Ecocrop, 2010; Ecoport, 2010). Banana leaves which have broad blades continuously grow from the center of the stem with a length of 1 – 4 meters and width of 0.7 – 1.0 meter (Hueze *et al.*, 2016). Banana leaves are a by-product obtained from banana production which are found in all tropical and subtropical regions of Africa, America, Asia and Australia where bananas are cultivated (Hueze *et al.*, 2016). According to (Ffoulkes *et al.*, 1977), banana leaves contain about 85 % moisture and 10 – 17 % protein. This indicates its suitability as a plant-based protein ingredient in fish feed. The Nile tilapia *Oreochromisniloticus* (Linnaeus, 1757) which belongs to the Cichlidae family is a very important aquaculture species in Nigeria. Some fish consumers prefer *O. niloticus* to other species due to its organoleptic properties such as high meat quality and nice taste after processing and preparation methods including sun drying, oven-drying, smoking, frying, barbecue, etc. Globally, *O. niloticus* is an important aquaculture species because of its fast growth rate, resistance to harsh environmental conditions and ease of fingerlings production under captivity (Gómez-Márquez *et al.*, 2003). Fecundity, egg size and gonadosomatic index are important parameters used in evaluating the reproductive ability and performance of fish. In aquaculture, fecundity is one of the criterion considered in selecting species for domestication (Mayer *et al.*, 1989). Gonadosomatic index in fishes indicates the gonad size ratio to fish size and could be used in evaluating the production of eggs (Amtyaz *et al.*, 2013; Eyo *et al.*, 2020). In Nigeria, high cost and availability of high quality fish feed is still a major constraint faced by farmers which may be attributed to high cost of conventional protein sources, such as shrimp and fishmeal (Awom and Eyo, 2016; Ekanem *et al.*, 2013; Eyo & Ekanem, 2011; Eyo *et al.*, 2014). To address this challenge, farmers are searching for cheaper and available sources of protein, most especially from plant-based origin such as leaf meals. Generally, leaf meals are among the cheapest source of protein that may significantly reduce the cost of fish feed production. One of the major challenge associated with the use of leaf meals is the presence of anti-nutritional substances including protease inhibitors, glucosinolates, phytates, saponins, lectins, tannins, oligosaccharides, non-starch polysaccharides, alkaloids, phytoestrogens, antigenic compounds, cynogens, gossypols, cyclopropenoid, mimosine, fatty acids, antivitamins, phorbol esters and canaranine (Francis *et al.*, 2001). These anti-nutritional substances could be reduced or eliminated through processing of the leaf meals before usage in feed production. The aim of this study was to evaluate the effects of Banana leaf meal (*Musa spp*) on the fecundity, egg size and gonadosomatic index of the Nile Tilapia (*O. niloticus*)

MATERIALS AND METHODS

Experimental design

This feeding trial was conducted for one hundred and twenty (120) days using a triplicate setup of six (6) tarpaulin tanks measuring (100 x 100 x 100 cm^3) labeled A_1, A_2, A_3, B_1, B_2 and B_3. Two isonitrogenous diets containing a crude protein level of 35 % (Diet A and Diet B) were used in feeding the experimental fishes. Diet A was the control diet and Diet B was formulated with the inclusion of Banana leaf meal (BLM) in addition to dietary feed ingredients (Table 1). A total of one hundred and fifty (150) fingerlings of *O. niloticus* with a mean body weight of 2.84 ± 0.11 g and mean total length 3.96 ± 0.12 cm were purchased from a private fish farm in Uyo, Akwa Ibom State and used for the study (25 fingerlings per tank). The fishes were acclimated for 14 days and during this period, the fishes were fed with Coppens feed to satiation. After the acclimation period, the fishes were starved for 24 hours before introducing the experimental diets to the fishes. Fish in tarpaulin A_1, A_2 and A_3, were fed Diet A (control diet) and fish in tarpaulin tank B_1, B_2 and B_3 were fed Diet B containing Banana leaf meal (BLM). Feeding was done twice daily at 5% of their body weight. Prior to the start of the experiment, biometric measurements including initial length and weight were taken for each tarpaulin tank using measuring board for length in cm and Metlar 5000D electronic scale for weight in grams.

Preparation of banana leaf meal (BLM)

Matured banana leaves were collected in fresh condition from banana trees in Calabar, Cross River State. After collection, the stalk was separated from the leaves and the leaves were washed with clean municipal water. The leaves were sundried with occasional turning until all the sundried leaves became brittle.

Dietary formulation (gkg^{-1}) and proximate composition (%) of experimental diets

The two isonitrogenous experimental diets formulated according to Pearson square method were composed of Soybean meal (SBM), groundnut meal (GNM), fish meal (FM), Neem leaf meal (*A. indica*), corn meal, vitamin premix, bone ash, sodium chloride (NaCl), vitamin C, wheat flour, lysine, calcium powder, methionine, and palm oil. All the ingredients including Banana leaf meal (BLM) were processed to powdery form before mixing and pelletizing using a locally fabricated mixer and pelletizer. Thereafter, the pelleted feed was sun-dried for 5 – 6 hours and stored at 4 °C in air tight sacs. For proximate composition of the experimental diets, methods AOAC (2005) of was adopted. Ash, crude protein, moisture content, lipid, crude fibre and carbohydrate were analyzed.

Table 1. Dietary formulation (gkg^{-1}) and proximate composition (%) of experimental diets

Ingredients	Diet A (Control)	Diet B (BLM inclusive)
Soybean meal (SBM)	193.60	179.40
Groundnut meal (GNM)	193.60	179.40
Fish meal (FM)	193.60	179.40
Bananna leaf meal (BLM)	--	179.40
Corn meal	359.2	222.40
Vitamin premix	10.00	10.00
Bone ash	5.00	5.00
Sodium chloride (NaCl)	5.00	5.00
Vitamin C	5.00	5.00
Wheat flour	10.00	10.00
Lysine	5.00	5.00
Calcium powder	5.00	5.00
Methionine	5.00	5.00
Palm oil	10.00	10.00
Total	1000.00	1000.00
Proximate Composition		
Ash (%)	5.73 ± 0.05^{a}	5.78 ± 0.11^{a}
Crude protein (%)	34.85 ± 0.10^{a}	34.53 ± 0.09^{a}

Moisture (%)	1.92 ± 0.07^{a}	1.95 ± 0.05^{a}
Lipid (%)	2.56 ± 0.04^{a}	2.45 ± 0.05^{a}
Crude fibre (%)	7.51 ± 0.05^{a}	7.73 ± 0.08^{a}
Carbohydrate (%)	47.33 ± 0.13^{a}	47.22 ± 0.13^{a}

*Means with the same superscript are not significantly different (P>0.05), BLM = Banana leaf meal

Plate 1: the experimental fish, Nile Tilapia (*O. niloticus*)

Plate 2: Banana leaves (*M. acuminata)*

Fecundity of *O. niloticus* fed experimental diets

Female fish collected from the two dietary treatments were dissected using a sharp scissor through the genital opening. Ovaries were carefully removed and washed in distilled water before weighing using Metlar-2000D electronic scale to the nearest 0.1 g according to (Ekanem *et al.*, 2017; Eyo *et al.*, 2013). To harden the eggs and loosen the surrounding ovarian tissues for easy counting, the eggs were fixed in Gilson fluid containing 17 ml of Nitric acid, 4 ml of Glacial acetic acid, 20 g of mercuric chloride, 95 % ethanol and 900 ml of distilled water (Ekanem *et al.*, 2017). Thereafter, fecundity (F) was determined according to Viveen *et al.*, (1985) as the product of weight of eggs in the ovary and count in 1 g of egg mass as follows:

$$Fecundity\ (F) = total\ weight\ of\ eggs\ in\ the\ ovary\ x\ count\ in\ 1\ g\ of\ egg\ mass$$

Measurement of egg size

For each female fish, the diameter of 40 eggs were measured using a stereo microscope with a micrometer ocular eye piece (Eyo *et al.*, 2013).

Gonadosomatic Index (GSI) of *O. niloticus* fed experimental diets

Gonadosomatic index (GSI) of *O. niloticus* fed the experimental diets was calculated following the equation of Bolger & Connolly (1989) follows:

$$GSI = (Gonad\ weight/Fish\ weight) * 100$$

Measurement of physicochemical parameters

Physicochemical parameters measured in this study included pH, temperature (°C), dissolved oxygen (mg/l) and ammonia (mg/l). pH was measured with Portable waterproof Hanna meter pH/EC/TDS (high range) - HI991301. Temperature was measured in °C using mercury in glass thermometer, dissolved oxygen was measured in mg/l using Portable Hanna dissolved oxygen meter Model HI9142 while ammonia in mg/l using ammonia test kit.

Statistical analysis

Data obtained for fecundity, egg diameter, male GSI and female GSI were subjected to T-test Analysis to determine if there were significant differences among fish fed the experimental diets using Predictive Analytical Software (PASW) version 18.0. Effects with a probability of (P> 0.05) were considered not significant.

RESULTS AND DISCUSSIONS

Fecundity and egg size of *O. niloticus* fed the experimental diets

Results obtained for fecundity of *O. niloticus* fed the experimental diets (Table 2) revealed that fish fed diet A (control) with a mean body weight of 104.0 ± 2.48 g and mean ovary weight of 3.30 ± 0.05 g had a mean fecundity of 868 ± 25.36 eggs. For *O. niloticus* fed diet B (BLM Inclusive) mean body weight was 101.50 ± 2.02 g, mean ovary weight was 3.18 ± 0.04 g and a mean fecundity of 863 ± 20.42 eggs. Fecundity which is the number of eggs in a gravid female fish has been documented by different authors to be influenced by diet composition and quality (Ekanem *et al.*, 2013; Eyo *et al.*, 2014; Eyo *et al.*, 2016). There was no significant difference (P > 0.05) in fecundity of fish fed the two experimental diets which indicates that diets were both of good quality. Quantity and quality of diets has been reported to affect spawning frequency and fecundity in tilapia (Little *et al.*, 2000). According to Ekanem *et al.*, (2013), utilization of high quality feed will enhance growth of ovaries and increase in fecundity of fish. Proximate composition of the experimental diets (Table 1) shows that the experimental diets contained the required nutrients for good health and optimal growth, egg formation and development of the experimental fishes. Findings of this study indicates that the inclusion of Banana leaf meal in the diets of *O. niloticus* did not impose any negative effect on the growth and fecundity of the fishes. Mean egg diameter (1.54 ±0.21 mm) of fish fed diet A (control) was not significant different (P > 0.05) from mean egg diameter (1.53 ±0.16 mm) of fish fed diet B (BLM Inclusive). This is attributed to the quality of the two experimental diets and also indicates that Banana leaf meal inclusion in the diets of *O. niloticus* has no negative effect on the egg size.

Table 2: Fecundity and egg size of *O. niloticus* fed the experimental diets

Indices	Diet A (Control)	Diet B (BLM Inclusive)
Mean fish weight (g)	104.00 ± 2.48[a]	101.50 ± 2.02[a]
Mean Ovary weight (g)	3.30 ± 0.05[a]	3.18 ± 0.04[a]
Mean fecundity	868 ± 25.36[a]	863 ± 20.42[a]
Mean egg diameter (mm)	1.54 ±0.21[a]	1.53 ± 0.16[a]

*Means with the same superscript are not significantly different (P>0.05), BLM = Banana leaf meal

Gonadosomatic Index (GSI) of *O. niloticus* fed experimental diets

Gonadosomatic index (GSI) shows the ratio of the gonad size that is relative to the size of fish and also indicates the level of gonadal development in fishes (Eyo *et al.*, 2020). According to Opeh *et al.*, (2018), gonadosomatic index (GSI) is a crucial tool used in aquaculture and fisheries to assess the reproductive state of fish. Results obtained for gonadosomatic index (GSI) of *O. niloticus*fedthe experimental diets (Table 3) showed that male fish fed diet A (control) with a mean body weight of 95.20 ± 3.44 g and mean gonad weight of 0.80± 0.02 g had a mean gonadosomatic index (GSI) of 0.84 ± 0.01 % while male fish fed diet B (BLM Inclusive) with a mean body weight of 93.50 ± 2.79 g and mean gonad weight of 0.79± 0.01 g had a mean gonadosomatic index (GSI) of 0.82 ± 0.02 %. Female fish fed diet A (control) with a mean body weight of 104.00± 2.48 g and mean gonad weight of 3.30± 0.05 g had a mean gonadosomatic index (GSI) of 3.18 ± 0.01 % while female fish fed diet B (BLM Inclusive) with a mean body weight of 101.50 ± 2.02 g and mean gonad weight of 3.23± 0.04 g had a mean gonadosomatic index (GSI) of 3.13 ± 0.04 %. In this study, there was no significant difference (P > 0.05) in the gonadosomatic index (GSI) and gonad weight of fish fed the two experimental diets. This shows that Neem leaf meal did not affect gonad development negatively in *O. niloticus.* This findings corroborates with the findings of Opeh *et al.*, (2018) that the inclusion of adequate level of leaf meal in fish diets did not affect gonad development and gonadosomatic index. In this study, female GSI are higher than male GSI and is attributed to bigger size of the female gonads (ovary) compared to the male and similar observation is found in almost all fishes.

Table 3: Gonadosomatic Index (GSI) of *O. niloticus* fed experimental diets

Indices	Diet A (Control)	Diet B (BLM Inclusive)
Mean male body weight (g)	95.20 ± 3.44[a]	93.50 ± 2.79 [a]
Mean male gonad weight (g)	0.80 ± 0.02 [a]	0.79 ± 0.01[a]
Mean male GSI (%)	0.84 ± 0.01[a]	0.82 ± 0.02[a]
Mean female body weight (g)	104.00 ± 2.48 [a]	101.50 ± 2.02[a]

Mean female gonad weight (g)	3.30 ± 0.05 [a]	3.23 ± 0.04[a]
Mean female GSI (%)	3.18 ± 0.03 [a]	3.13 ± 0.04[a]

*Means with the same superscript are not significantly different (P>0.05), BLM = Banana leaf meal

Physicochemical parameters of the tarpaulin units

Mean physicochemical parameters of the tarpaulin units measured in this study including pH, water temperature (ºC), dissolved oxygen (mg/L) and ammonia level (mg/L) were within the optimum range recommended for optimal growth and health of freshwater fishes according to Boyd (1979).

Table 4: Physicochemical parameters of the tarpaulin units

Indices	Diet A (Control)	Diet B (NLM Inclusive)
Mean pH	6.95 ± 0.05[a]	6.97 ± 0.03[a]
Mean water temperature (ºC)	29.65 ± 0.35[a]	29.68 ± 0.30[a]
Mean dissolved oxygen (mg/L)	4.98 ± 0.20[a]	4.87 ± 0.23[a]
Mean ammonia level (mg/L)	0.001 ± 0.001[a]	0.001 ± 0.001[a]

*Means with the same superscript are not significantly different (P>0.05)

Conclusions and recommendations

In conclusion, the inclusion of Banana leaf meal (*M. acuminata*) in the diet of *O. niloticus* did not impose any negative effect on the fecundity, egg size and gonadosomatic index (GSI). However, the inclusion of Banana leaf meal (*M. acuminata*) in the diet of *O. niloticus* resulted in a reduction in the quantity of other conventional feed ingredients such as soybean meal (SBM), fish meal (FM), groundnut meal (GNM) and corn meal (CM). For a cost effective production of *O. niloticus*, the inclusion of Neem leaf meal (*M. acuminata*) in the fish diet is therefore recommended.

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