EFFECT OF WONDERFUL KOLA SEED MEAL (*Buchholzia coriacea*) ON GROWTH, MASCULINIZING POTENCY AND GONADAL GROSS MORPHOLOGY OF THE NILE TILAPIA (*Oreochromis niloticus*, LINNAEUS 1758)

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Abstract

This study was aimed to investigate the effect of *Buchholzia coriacea* on the masculinization and growth of *Oreochromis niloticus*. The larva of *O. niloticus* (mean weight 0.2g, mean length 0.45mm) were subjected to powdered *B. coriacea* seeds meal (BSM) at 0, 2, 4, 6, 8, 10 g/kg twice daily for one month in an indoor experimental plastic basin in duplicates with a total of six treatments. After which they were later fed with a normal diet in an outdoor concrete tank for another one month. The result obtained in this study showed that fish fed with 4g/kg BSM had the highest number (83.33%) of males which was significantly higher ($p<0.05$) compared to all other treatments categories. The highest survival rate (100%) was recorded in fish fed with 6g/kg BSM. The mean weight and weight gain observed are 4.68 g, and 4.65 g respectively were recorded for fish fed with 4g/kg BSM. The result of the histology showed that *B. coriacea* altered the sex of *O. niloticus* due to the presence of a hormone called dopamine in the kola. Conclusively, *B. coriacea* showed potential reproduction control in *Oreochromis niloticus*.

Keywords : *Buchholzia coriacea*, Growth, Masculinization, *Oreochromis niloticus*, Traditional herb

INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) belonging to the family Cichlidae, is one of the most popular tilapia species cultured around the world. *O. niloticus* is very suitable for aquaculture because of its several favorable culturable characteristics such as high fecundity, fast growth, appreciable size, disease resistance, tolerance of harsh environmental conditions, acceptability of formulated feed, high meat quality, high market value, and good taste. Apart from its promising culture characteristics, *O. niloticus* has been used in many areas as biological control of disease vectors of diseases such as bilharzia, malaria, and zika through predation on the hosts of the parasites. On a global scale, *O. niloticus* has rated the 4th most cultured fish species with 2,537 tons in 2010, 3,260 (2012), 3,677 (2014) and 4,200 tons (2016) representing 8 % of the major species produced world aquaculture (FAO, 2018).

In Africa, *O. niloticus* is responsible for almost half (43.6%) of aquaculture production with other freshwater species such as African catfish (*Clarias gariepinus*) contributing 11.9 % and common carp contributing 10.5 % (FAO, 2018).

Buchholzia coriacea which is commonly also as wonderful kola belongs to the Capparaceae family and it has been used traditionally for medicinal purposes (Mbata *et al*., 2008). In Nigeria, it is named according to tribes such as Uke (Igbo), Owi (Edo) and Uworo (Yoruba). *B. coriacea* plant parts including leaves, roots, seeds, and stem are used to increase male sex hormone and their concoctions are useful in treating ailments caused by pathogenic microorganisms including fever, malaria, menstrual and gastrointestinal infections (Amaechi, 2009; Ezeji *et al*., 2011). In 2016, global fish production peaked around 71 million tonnes, in which aquaculture represented 47 % of the total and 53 % if non-food uses including a reduction to fish oil and fishmeal are excluded (FAO, 2018). With the relative static production in capture fishery since the late 1980s, sustainable and consistent supply of food fish for human consumption is attributed to aquaculture (FAO, 2018).

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Despite the increase in popularity of tilapia aquaculture, its growth is hindered by their early maturation and proliferated breeding. These problems result in overcrowding of the pond due to an overpopulation of the fish which consequently leads to poor or stunted growth and deterioration of pond water quality (Fashina-Bombata and Busari, 2003). Significant differences in growth of individual attributed to sex have serious consequences for production, including unsalable small fish and variable harvest weight (Lind *et al.,* 2012). Another challenge associated with tilapia culture is sizes of fish at harvest which varies from small to large attributed to fast growth rate and better feed conversion of the male than female. Due to the superior growth rate of males coupled with available sex-reversal techniques, the production of all-male populations is envisaged by a significant proportion of the tilapia aquaculture industry to control the reproduction and improve productivity (Mair *et al*., 1997).

The magnitude or degree of sexual size dimorphism (SSD) of tilapia now becomes a determinant factor controlling the advantageous growth rate of mono-sex over mixed-sex populations (Lind *et al*., 2012). One of the most common and frequently used approach to eliminate these problems and control reproduction in tilapia is the monosex male culture strategy. This strategy was first developed in Japan in the 1950s for reversing the sex of aquarium fish and carp species and commercialized in the 1970s (Baroiller and D'Cotta, 2000). In fish, control of reproduction requires gametes manipulation (Mohammadi *et al.,* 2015).

However, basic knowledge on fish reproduction physiology is necessary for developing effective artificial insemination techniques, (Billard and Jensen, 1996). Nowadays, various techniques have been successfully used in controlling reproduction in tilapia such as manual sexing, hybridization, chromosomal manipulation, and hormonal sex reversal (Bentsen *et al*., 2012; Megbowon and Mojekwu, 2013).

The use of sex reversal hormonal such as 17 α-methyltestosterone is seen as the most reliable and simple method of producing all-male tilapia stock, which grows larger at a uniform and faster rate compared to mixed-sex culture (Megbowon and Mojekwu, 2013). However, this technique and other masculinizing technologies are highly expensive, require technical skills, and may not be easily understood and applied by local fish farmers.

Hormonal sex reversal may result in serious environmental issues, fish and public health implications on the treated fish and fish consumers due to the presence of steroids. Also, manual sexing which involves intensive labor may expose fish to handling stress and any sexing error will result in mixed-sex culture. The objective of the present study is to evaluate the effect of wonderful kola seed meal (*B. coriacea*) on growth, masculinizing potency and gonad gross morphology of the Nile tilapia (*Oreochromis niloticus*).

METHODOLOGY

Place and Time

The study was carried out in the Fish Hatchery Complex of the Department of Fisheries, University of Maiduguri, Nigeria. Geographically, it is located at Latitude11.80 °N and Longitude 13.19 °E with an elevation 325 meters above sea level.

Research Materials

The experimental feed was composed of the following ingredients: *B. coriacea* seed, soybeans, wheat bran, trash fish, vitamin C, Vitamin Premix, starch, methionine, and starch. The ingredients were procured from Baga market in Maiduguri. The feedstuffs, including soybeans, wheat bran, and trash fish were separately milled to obtain a fine powder. Soybeans were toasted at 80ºC for 30 minutes before milling. *B. coriacea* seeds

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were soaked in water for 24 hours to remove contaminants before air drying. After that, the air-dried grains were milled to fine powder and store in a cold, dry place.

Research Design

The experiment was set up in a complete randomized design with a total of 6 treatments in duplicate. Sixty (60) 5-day old fry with a total weight range of 0.1 – 0.3g and total length range of 4 – 5 mm were stocked in twelve (12) 30 liters capacity trough. The troughs were labeled A1, A2, B1, B2, C1, C2, D1, D2, E1, E2, F1, and F2. Fries stocked in trough A1 and A2 were fed Diet 1 (control) containing 0 % of BSM, B1 and B2 were fed Diet 2 (2% BSM), C1 and C2 were fed Diet 3 (4% BSM), D1 and D2 were fed Diet 4 (6% BSM) E1 and E2 were fed Diet 5 (8% BSM), and F1 and F2 were fed Diet 6 (10% BSM) respectively.

Feeding was done twice daily ad-libitum for 30 days. Uneaten feed was siphoned every morning before feeding the fries to avoid deterioration of the water in the experimental basin. At the end of the 30 days feeding period, the fish were transported into 12 ($1m^3$) outdoor concrete pond with the same label given to the trough for another 30 days feeding period before assessing the sexes. During this period, the fish were fed a regular diet of 30% CP without *B. coriacea* Seed Meal (BSM).

Work Procedures

Proximate Analysis of the Experimental Diets

Proximate composition of the six experimental diets was analyzed following standard methods and procedures given by AOAC (2000).

Determination of the Sex of *O. niloticus* Fed Experimental Diets

At the end of the 30 days feeding period in the outdoor ponds, the sexes of the fish were identified manually with the aid of the gentian violet and cotton. The cotton wool was soaked into gentian violet, and a smear was applied on the genital papilla to see the openings for male or female. The numbers of male and female were recorded.

Table 1. Feed composition of the experimental diets.

Ingredients (%)	Diet A (0 % BSM)	Diet B (2 % BSM)	Diet C (4 % BSM)	Diet D (6 % BSM)	Diet E (8 % BSM)	Diet F (10 % BSM)
Wheat Bran	45.36	45.36	45.36	45.36	45.36	45.36
Fish Meal	21.67	21.67	21.67	21.67	21.67	21.67
Soya Bean	21.67	21.67	21.67	21.67	21.67	21.67
Premix	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin C	0.05	0.05	0.05	0.05	0.05	0.05
Salt	0.3	0.3	0.3	0.3	0.3	0.3
Methionine	0.35	0.35	0.35	0.35	0.35	0.35
Lysine	0.3	0.3	0.3	0.3	0.3	0.3
Starch	10	8	6	4	2	0
B. coriacea seed meal	0	2	4	6	8	10
Total	100	100	100	100	100	100

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Gonad Gross Morphology of the Experimental Fish

Gonad gross morphology of the experimental fish was evaluated using histological analysis according to Eyo *et al.* (2014). Gonads of male and female fish (testes and ovaries) fed the six experimental diets were collected processed following the stepwise procedure reported by Brown and Peterson (1911).

The collected gonads were fixed in Bouin's fluid for 4 days before dehydration in alcohol (30 %, 50 %, 70 %, 90 %, and 100 %). After that, the tissues were cleared in chloroform/xylene solution (50:50) before clearing in pure xylene. The cleared tissues were impregnated in wax before blocking out and sectioning using a rotary microtome at 10 µm. After sectioning, the tissues were stained using hematoxylin and eosin stain (H&E) before photomicrograph using Amcap Microscope Camera, Model: DCE – 2.

Measurement of Water Quality Parameters

Water quality parameters including pH, dissolved oxygen (mg/l) and water temperature were measured weekly in each replicate unit of the experiment. pH was measured using PHEP pH meter, dissolved oxygen was measured using oxygen meter, and the water temperature was measured using mercury in glass thermometer.

Data Analysis

Data obtained from the experiment were subjected to one-way analysis of variance (ANOVA) using Predictive Analytical Software (PASW) version 19. Means were separated using the least significance difference (LSD). Effect with a probability of $P<0.05$ were considered significant.

RESULTS AND DISCUSSION

Sex reversal of *O. niloticus* Fed Diets with Varying Levels of *B. coriacea* seed meal (BSM)

Results obtained for the sex reversal (Table 2) of *O. niloticus* fed diets with varying levels of *B. coriacea* seed meal (BSM) showed that the highest number of males (83.33 ± 3.33 %) was recorded in fries fed Diet C (4 g/kg BSM), followed by 74.99 ± 1.66 % in fries fed Diet E (8 g/kg BSM) and Diet F (10g/kg BSM), followed by 73.33 ± 3.33 % in fries fed Diet D (6 g/kg BSM), followed by 54.99 ± 1.66 % in fries fed Diet B (2 g/kg BSM) and least (51.66 ± 1.66 %) in fries fed Diet A (Control).

The least number of females (16.66 ± 3.33 %) was recorded in fries fed Diet C (4 g/kg BSM), followed by 24.99 ± 1.66 % in fries fed Diet E (8 g/kg BSM) and Diet F (10g/kg BSM), followed by 26.66 ± 3.33 % in fries fed Diet D (6 g/kg BSM), followed by 44.99 ± 1.66 % in fries fed Diet B (2 g/kg BSM) and highest (48.33 ± 1.66 %) in fries fed Diet A (Control).

Table 2. Sex reversal of *O. niloticus* fed diets with varying levels of *B. coriacea* seed meal (BSM).

Sex	Diet A (0 % BSM)	Diet B (2 % BSM)	Diet C (4 % BSM)	Diet D (6 % BSM)	Diet E (8 % BSM)	Diet F (10 % BSM)
No. of Males (%)	$51.66±1.66^a$	$54.99±21.66^a$	$83.33±3.33^a$	$73.33±3.33^a$	$74.99±1.66^a$	$74.99±1.66^a$
No. of Females (%)	$48.33±1.66^a$	$44.99±21.66^a$	$16.66±3.33^a$	26.66±3.33a	24.99±1.66a	24.99±1.66a

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Water Quality Parameters of the Experimental Units

Results of water quality parameters (Table 3) showed that the highest temperature (28.25±0.05 ºC) for the indoor tank was recorded in experimental plastic tank one and the highest temperature for the outdoor tank (28.25±0.15 ˚C) was recorded in concrete tank 2. The lowest temperature (26.65 ± 0.25 ˚C and 27.65±0.65 ˚C) was recorded in experimental plastic tank 3 and concrete tank 4 (outdoor) respectively. For pH, the highest value for indoor and outdoor tanks (8.47 ± 0.03) was recorded in plastic tank 4 and concrete tank 4 respectively. The lowest pH value of for indoor and outdoor tanks (8.33 ± 0.07) was recorded in plastic tank 6 and concrete tanks 1, 2, and 3.

The highest value of dissolved oxygen for indoor and outdoor tanks (5.57 ± 0.07 mg/l and 5.75 ± 0.05 mg/l) was recorded in plastic tank 2 and concrete tank 1 and 4 respectively. The lowest value of dissolved oxygen for indoor and outdoor tanks (5.44 ± 0.03 mg/l and 5.55 ± 0.05 mg/l) was recorded in plastic tank 1 and concrete tank 2.

Table 3. Mean water quality parameter for both indoor and outdoor experimental tanks.

Experimental Tank	Indoor			Outdoor		
	Temperature (ºC)	pH	DO (mg/l)	Temperature (ºC)	pH	DO (mg/l)
0	26.75±0.25^{a}	8.45±0.01^{a}	5.63±0.65^{a}	28.50±0.5^{a}	8.33±0.01^{c}	5.75±0.05^{a}
2	28.25±0.05^{a}	8.40±0.25^{b}	5.57±0.07^{ab}	28.65±0.15^{a}	8.33±0.03^{c}	5.55±0.05^{c}
4	27.85±0.05^{a}	8.37±0.08^{c}	5.56±0.01^{ab}	27.65±0.65^{a}	8.33±0.03^{b}	5.56±0.01^{abc}
6	27.00±1.00^{a}	8.45±0.01^{a}	5.44±0.03^{b}	28.50±0.80^{a}	8.47±0.03^{b}	5.75±0.05^{a}
8	27.75±0.75^{a}	8.47± 0.03^{ab}	5.61±0.05^{ab}	28.15±0.35^{a}	8.45±0.00^{b}	5.60±0.00^{bc}
10	27.55±0.75^{a}	8.33±0.03^{c}	5.61±0.05^{a}	28.15±0.35^{a}	8.45±0.01^{a}	5.60±0.00^{ab}

Note : means with the same superscript are not significantly different (P>0.05)

Sex Reversal

B. coriacea seed meal (BSM) has been reported to significantly increase Luteinizing Hormone (LH), testosterone, Follicle Stimulating Hormone (FSH), elevator anti muscle weight, prolactin hormone levels, motility and sperm count in infertile obese mutant rat models (Kumar, 2002). Additionally, *B. coriacea* seed meal (BSM) has been used to increase libido in men due to its dopamine inducing properties (Shukla *et al.,* 2010; Giuliano and Allard, 2001). The results of this study showed that each treated group gave a mean male/female ratio that deviate significantly from the normal 1:1 ratio, with a significantly higher (P<0.05) male ratio compared to than females.

The success of sex-reversal experiments is usually evaluated using a percentage of males which is to a large extent dependent on the intake feed treated with the hormone (Baroiller *et al.*, 1995). According to Mair and Little (1991) and El-Sayed (2006), hormone administration through feed intake is influenced by several factors including treated feed quality, feed ingredients composition and processing methods, feed storage, ingredients particle size, and feed palatability. The control group was very close to the normal 1:1 ratio. This indicates that the variation in male to female ratio in fish fed *B. coriacea* supplemented diets compared to the control fish observed in this study could be due to the effect of *B. coriacea* seed meal (BSM) present in the diets.

Adhikari (2019) reported the efficacy of *B. coriacea* seeds in the treatment of

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several sexual asthenia and infertility in human implying that it contains androgens.

Also, the methanol extract of its leaves stimulated testosterone production in testicular fractions and Leydig cell cultures in the average adult albino male rat. In this study, none of the feed containing varying levels of *B. coriacea* seed meal (BSM) gave 100 % male population of *O. niloticus* implying that *B. coriacea* seed is not efficient in tilapia sex reversal. The use of steroids as masculinizing agents in tilapia culture has been reported to be 90-100% efficient in *Sarotherodon niloticus, O. mossambicus* and *O. niloticus* (Tayamen and Shelton 1978; Pandian and Koteeswaran, 1998).

These results are higher than 83.33% males recorded in this study for *O. niloticus* fed Diet C (4 % BSM). The highest number of males 83.33 % recorded in this study for *O. niloticus* fed Diet C (4 % BSM) is lower than 92 % of males reported by Chukwu and Shaba (2009) after treating *O. niloticus* with 8g/kg of pawpaw seed for 60 days. The 83.33% males *O. niloticus* obtained in this study could be attributed to the time of commencement of experimental diet administration which was from one-week-old fry weighing 0.2g.

However, these findings are better than 73.33 % obtained by Mukerjee *et al*. (2015) after feeding *O. niloticus* with diets formulated with *Mucuna puriens* seed meal at 5.0g/kg for 40 days. Interestingly, fish fed diet D (6 g/kg BSM) recorded the same percentage (73.33 %) for males obtained by Mukherjee *et al.,* (2015) after feeding *O. niloticus* with a diet containing at 5.0g/kg of *M. puriens* seed meal.

Findings of this study are however, higher than the findings of Okoko (1996), who obtained 71.9 % males at the dose rate of 120 mg kg-1 MT of feed. The results of this study showed a significantly lower (P<0.05) male proportion of 74.99% for the highest dose rate of *B. coriacea* seed meal of 10g/kg. These results are in line with the findings of Okoko (1996), who obtained 99.3 % males at 30 mg MT, while 97 and 71.9 % males at the dose rates of 60 and 120 mg MT, respectively. These findings imply that a higher dose rate of *B. coriacea* seed in *O. niloticus* diets did not yield to a higher percentage of male. 4g/kg BSM recorded the highest male production because it was included in the optimum quantity.

Gonad morphology

In this study, the histology of female gonads of *O. niloticus* treated with *B. coriacea* seed showed no sign of gonads development. This is because the number of days *O. niloticus* fry was fed with *B. coriacea* seed meal was relatively low (30 days) compared to Jegede and Fagbenro (2008) in a related study of *O. niloticus* fed with varying levels of *C. papaya* which lasted for 60 days. The features of the male testis are still developing; hence, no effect was observed. Thus, if the period for 30 days of feeding *O. niloticus* with *B. coriacea* based feed is prolonged, there is a tendency that the ovaries and the testis may disintegrate.

CONCLUSION

The result emanating from this study can be used as an alternative method to produce all-male tilapia population in an environmentally friendly manner using a natural product. However, the inclusion of BSM at the recommended quantity has no adverse effect on the gonad morphology of fish.

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