



GROWTH RESPONSE, SEX RATIO AND ECONOMICS OF MASCULINIZED *Clarias gariepinus*



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Abstract: The growth response, sex ratio and economics of masculinized *Clarias gariepinus* using lyophilized bull and goat testes meal was evaluated. Masculinization of *Clarias gariepinus* was conducted using Methyl testosterone Hormone-treated diet (MTH), Lyophilized Bull Testis Meal (LBTM) and as well as Lyophilized Goat Testis Meals (LGTM) for four weeks. Lyophilization of the testes obtained from Kano abattoir was done following standard procedures. 1.5 mg/l MTH was mixed with 1Kg of Copens fish starter feed. The hatchlings were fed with the experimental diets for four weeks. The growth response and economics of masculinized *C. gariepinus* were evaluated. Results indicated that water temperature range during the experiment was (26.23-26.95°C), 7.55-7.60 for pH, 5.10-5.60 mg/l for dissolved oxygen were within appropriate ranges for good growth of *C. gariepinus*. Growth performance *C. gariepinus* indicated that fish fed lyophilized bull testes meal (LBTM) had the highest weight gain (1252.26 g) as well as length gain of 24.33 cm, this was followed by lyophilized goat testis meal fed treatment (LGTM) with 1188.67 g weight gain compared to commercial feed. The cost and return analysis of masculinization of *C. gariepinus* indicated that the lowest masculinization cost (N31, 430.00) as well as lowest net income (N43, 967.42) was obtained from control group, LBTM had N34, 930.00 masculinization cost and net income value of N64, 766.13 being the highest income obtained, LGTM had N34, 869.13 masculinization cost as well as N36, 930.00 income cost. MTH group had the highest masculinization cost of N41, 930.00 and income of N48, 565.77. The cost of masculinization compared with the net income shows that LBTM showed better output with the net income of N64, 700.13 while MTH (48,565.47) had the least net income. Conclusively, LBTM masculinized *C. gariepinus* gives better growth and economic returns, therefore should be adopted by fish farmers for to boost growth of *C. gariepinus*.

Keywords: Masculinization, Goat testes meal, Bull testes meal, *Clarias gariepinus*

Introduction

The total aggregate domestic fish supply from all sources (capture and culture fisheries) is less than 0.7 million metric tons per annum (Atanda, 2012), hence, Nigeria has to import about 0.7 million metric tons of fish valued at about \$500 million annually to augment the shortfall. In 2009, about N97 Billion was spent importing fish into Nigeria. This massive importation of frozen fish in the country has ranked Nigeria the largest importer of frozen fish in Africa. The huge sum of money spent by Nigeria annually in fish importation could be used to invest in fish farming.

Feed and feeding cost are the major concerns of any animal farmer. Feed covers the portion of production cost in commercial fish and livestock farms (Buyukcopar and Kamalak, 2007). Feed takes the principal portion of operation cost in the World of fish production and mainly represented in the form of fishmeal as a traditional vital component of both fish and animal feed (Glencross *et al.*, 2007). Masculinization in fish is an important economic tool which facilitates faster body growth and development by expending energy slated for egg production, reproduction and breeding to body growth (Sayeed, 2007). The use of methyl testosterone hormone in fish sex-reversal is believed to facilitate gonadal degeneration thereby causing either sterility in some fish species or change sex of others to male which are all considered to be of positive advantage in fish production. This is because the energy for reproduction is now converted to body muscle production in form of growth and development (Khalil *et al.*, 2011).

However, steroid treatment may have the disadvantages of being costly. It also sometimes causes production of sterile populations and occasional paradoxical feminization with prolonged exposure at stages of gonadal development in the African catfish (Turan, 2005).

Production of all-male fish (e.g. tilapia and *Clarias*) populations through administration of artificial and natural hormones is very effective and assisted in production cost

reduction thereby creating a good economic advantage to the farmer in terms of reduced production cost (Phelps and Popma, 2000). Al-ablani and Phelps (2002) also reported that exogenous steroids can be effective in controlling fish sexual development thereby enhancing economic body growth. Investigation into possible substitution of steroids with locally available and natural hormone sources may give a good solution to the fear researchers have on the androgens. The use of the Mammalian testes such as goat and bull may be a very good milestone is looking for an alternative to androgens, this necessitate the evaluation of the growth response, sex ratio and economics of masculinized *Clarias gariepinus*.

Materials and Methods

Lyophilization and experimental feed preparation

The bull and goat testes meals were prepared according to the methods of Phelph (2000); Fashina-Bombata & Somotun (2008) and Odin & Boliver (2011). Fresh testes were obtained from municipal abattoir in Kano City. The testes were immediately covered with multiples of polythene bags and stored in thermos-regulatory flask filled with ice crystals and covered completely. The testes were then taken to the Central Laboratory, Bayero University, Kano. The testes were immediately skinned and freed from epididymis, sliced and completely homogenized without dilution using Binatone blending electronic machine model ep35. Testes from each group of animals were kept separately i.e. goat and bull. Ages, weights and health status of all the animals were ascertained by the livestock health officer in the abattoir. This was making sure that the animals were matured and healthy. The creamy coloured

homogenized testes solution was then poured into the lyophilizer cups and placed in a freezer, then allowed to freeze for 24 h. The low processing temperature and absence of water help to maintain the colour, flavour and texture of the samples. Between 20-25% weight of the testes was regained. The crumbles were pulverized, sieved and stored in plastic containers and labeled according to treatment prior to feeding of fish.

Methyl Testosterone Hormone (MTH) supplemented commercial feed as control diet was prepared using 3 g of MTH dissolved in 2 litre of 95% ethyl alcohol (Atanda, 2012). The mixture was mixed thoroughly to get a clear solution, 200 ml of the stock solution was mixed with 1 kg Copens starter feed under highly aerated condition and spread to allow the alcohol evaporates following the method of Popma & Green (1990), Green and Teichard-Codington (2007). This feed was allowed to dry and stored in a safe container. This feed was the MTH feed used as Diet A.

Determination of proximate composition and testosterone concentration in experimental feed

Proximate analyses, crude protein, moisture content, ash, crude lipid, crude fibre and Nitrogen Free Extract were carried out following standard laboratory procedure as described in AOAC (2000). Analysis was conducted in the Biochemical Laboratory, National Research Institute for Chemical Technology, (NARICT), Zaria. High Performance Liquid Chromatography (HPLC) was employed in the determination of the testosterone levels in the masculinized *Clarias gariepinus* fish. The method of Barbosa *et al.* (2013) was employed in the HPLC, 17 α -methyltestosterone as synthetic steroid normally used in fish sex conversion in young fish. The procedure involved determination of methyl testosterone following Solid-Phase Extraction (SPE) using Ultra-Violet light detector. Shimadzu HPLC system model LC-2010 HT was used. The Muscle Sample was digested and subjected to chromatographic filtration through a reverse phase column (RP-C₁₈). The column filters the sample down to 5 micro metre size (5 μ m) and 250 mm by 4.6 mm at 25°C. Automatic injection was performed through 20 μ l loop on the automatic sampling unit then analytical detection was done using Ultraviolet (UV) light detector at 245nm and ultrapure water at 45:55v/v were used in the separation of analyte at running mobile phase at 1ml/minute flow rate at 25°C. This gives a good result; the sample was then optimized and cleaned using DSC-18um at 6 ml/500mg centrifuge.

Brood stocks Collection and Management

The brood stock (parents) fish was sourced from Fish House Fisheries Ltd, Isa Kaita Road, Kaduna and were transported in 2 black (50 litres) Jerry cans to Hatchery Room, Skills Acquisition and Development Centre, NAERLS, ABU, Zaria. Two, one thousand litre plastic containers were used to keep the parent according to sex. Brooders were fed commercial feed a body weight according to Bolorunduro (2002). Water quality parameters such as water temperature, pH and dissolved oxygen (DO) were also monitored.

Hatching procedures

The brooders were selected, acclimatized for one week and kept in separate tanks based on their sexes in the fish hatchery. Ovaprim hormone was administered intramuscularly in the female for ovulation at 0.5 mg/kg, and eggs were striped and fertilized artificially. The hatchlings were allowed to swim and absorb yolk within 2 days. The larvae were transferred to the nursery plastic tank and were monitored under controlled water quality parameters (temperature, pH, and dissolve oxygen) for 48 hours before they were stocked according to the experimental design. The experimental diets were administered for 28 days (Odins & Bolivar, 2011), after

28 days a commercial feed was introduced, and the fry were fed for five months.

Determination of experimental fish carcass composition

Artificially bred fries of *C. gariepinus* were masculinized through feeding using lyophilized bull and goat testes meal (LBTM and LGTM), after attaining post juvenile stages, fish were fed commercial diet for twenty weeks. Fish were harvested and processed. They were washed and oven dried at 60°C for a period of 72hrs after which they were pulverized and stored in plastic containers for further analyses. Proximate analyses was carried out as described by the Association of Official Analytical Chemist (AOAC, 2000), amino acid analyses was carried out according to the standard method of Robyt & White (1990).

Experimental design

The experiment was designed in two phases. Phase one was the hatchery activity phase. It involves dividing the fish into four experimental treatments (A, B, C and D). Each treatment was triplicated. Treatment A was fed feed containing MTH, treatment B was fed lyophilized bull testes meal (LBTM) and treatment C was also fed lyophilized goat testes meal (LGTM), and treatment D was the control treatment, fish at the this phase were fed commercial diet (0.2mm copens) for 28 days. The fish were stocked in 12 plastic containers of 1000liters capacity, each container contained 50 fish, and feeding of fish continued for five months with coppen.

Growth determination

Fish were weighed fortnightly using an electric Mettler Zurich (Swiss made) top-loading balance model CP8201.

Cost and returns analysis of masculinization of *Clarias gariepinus*

Economics analyses were computed as reported by Adams *et al.* (2014):

GP=TR-TC Where; GP = Gross Profit, TR = Total Revenue (₦), TC = Total Cost (₦)

Economic viability:

Profit Index= Value of fish (N) / cost of feed (N)

Incident of Cost=Cost of feed (N) / mean weight gain of fish produced (g)

Net Profit = Total Cost of Fish cropped (N)-Total Expenditure (N)

Benefit Cost Ratio = Total Cost of Fish cropped (N) / Total Expenditure (N)

Data analyses

Data collected were analyzed using one way Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) was used to separate mean where significant, at $p \leq 0.05$ using SAS 2007 version 9.1.3 software.

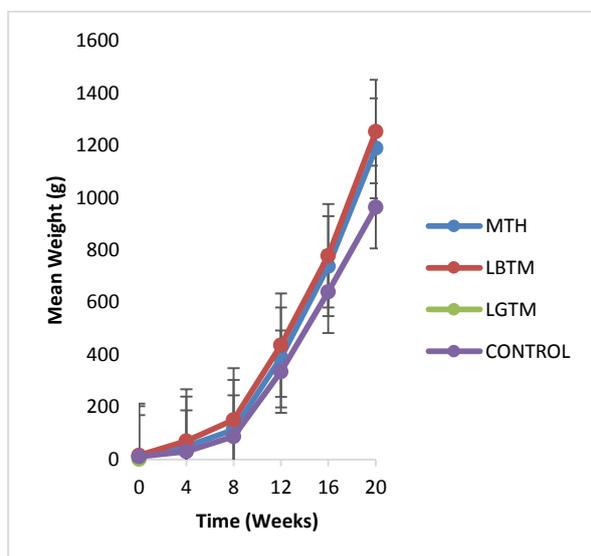
Results and Discussion

Table 1 Proximate compositions (%) and testosterone level of experimental diets used in the masculinization of *Clarias gariepinus* for four weeks. Diet A (Copens fish starter with MTH), diet B was Lyophilized Bull Testes Meal (LBTM), C was Lyophilized Goat Testes Meal (LGTM) and diet D was Copens starter only. The result revealed that in the four diets (i.e. A, B, C, and D), the moisture contents ranged from 1.45 \pm 0.01 (diet A and D) and 2.14 \pm 0.01 (diet C), diet B and C had higher moisture content than diet A and D. The ash content of the four diets were 9.9 \pm 0.50, 9.13 \pm 0.00, 8.88 \pm 0.01 and 10.20 \pm 0.01 for diet A, B, C and D respectively. Diet D had the highest ash content and diet B had the lowest ash content. Diet B had the highest crude lipid content (15.61 \pm 0.00), significantly higher than diets A and D (13.00 \pm 0.00 and 13.00 \pm 0.00), while diet C closely the same with diet A. Crude protein of all the experimental diets was generally high. Diet

B consisting of LBTM (71.12± 2.20) was the highest followed by diet C (69.31± 1.50), diets A and D had 59.12± 1.20 and 58.00± 1.10, respectively lower than the two values mentioned. Nitrogen Free Extract (NFE) values of the diets indicated that diet D had the highest value (16.22± 0.50) followed by diet A (16.03±0.60), diets B and C had the lowest values of 2.14± 0.02 and 4.40± 0.00 respectively. The Crude fibre contents of the diets were very low. Diets A and D had the highest value (0.05± 0.00 each), while diets B and C had lowest CF values of 0.00±0.00. There was significant difference in the proximate composition of the four diets ($P\leq 0.05$). The testosterone level (Table 4.2) of LBTM (14.12) and LGTM (8.91) differed significantly ($P\leq 0.05$).

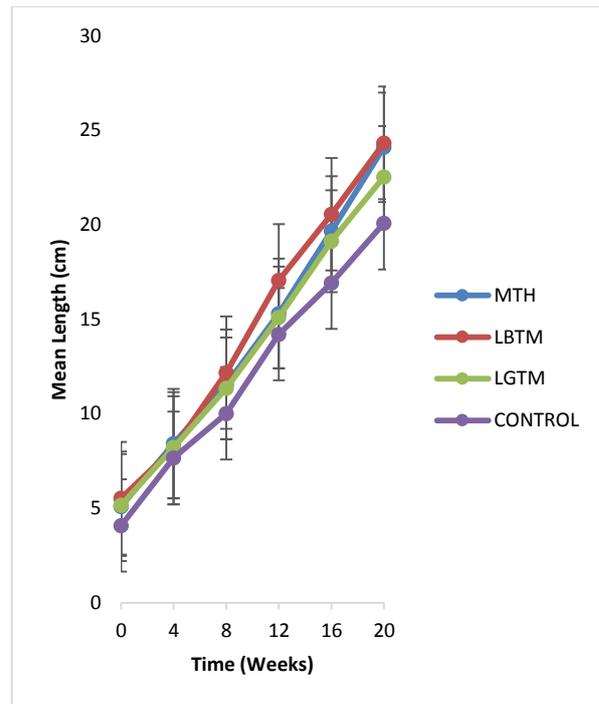
The crude protein of LBTM and LGTM were slightly the same to that of bull and boar 71.69 and 70.20 g/100g respectively as reported by Odin and Bolivar (2011), used lyophilized caraboar, bull and boar testes meal to masculinize Tilapia fish. Moisture content recorded in this study were lower than that reported by Odin and Bolivar (2011), this could be attributed to the level of Lyophilization, and the lower moisture content of the testes in this study could increase the shelf-life. The crude lipid were slightly higher than lyophilized caraboar, bull and boar testes meal reported by Odin and Bolivar which ranged between 12.33-13.59 g/100g. The ash content were slightly the same, testosterone concentration in lyophilized bull testes recorded in this findings was higher than caraboar, bull and boar used in masculinization of tilapia as reported by Odin and Bolivar (2011), these differences could be attributed to the serum sample used in determining the testosterone level in the animal while in this study tissue sample from testes were used.

Figure 1 shows the growth response of the fish during the experiment. Treatment A, B, C and D indicated slow increase at the beginning of the experiment. Treatment B (LBTM) had the highest total weight gain of 1252.27±23.07 g. This was followed by treatment A (MTH) which had the total weight gain of 1188.67± 0.35 g, Treatment C (LGTM) 1170.23± 23.06 g and group feed only coppers starter; Treatment D (Control) had the lowest weight gain of 963.70±23.06 g, in comparison treatments A which contains methyl testosterone hormone and B containing lyophilized bull testes meals did not show significant difference in values but differ significantly ($P\leq 0.05$) with treatment D (the control) in which the meal was commercial fish feed and these differences could be attributed to the testosterone levels in the experimental diets (Odin and Bolivar, 2011).



MTH= Methyl Testosterone Hormone; LBTM=Lyophilized Bull Testes Meal; LGTM= Lyophilized Goat Testes Meal

Fig. 1: Mean weight of MTH, LBTM and LGTM masculinized *Clarias gariepinus*



MTH= Methyl Testosterone Hormone; LBTM=Lyophilized Bull Testes Meal; LGTM= Lyophilized Goat Testes Meal Control-Control Treatment

Fig. 2: Mean Standard Length of MTH, LBTM and LGTM masculinized *Clarias gariepinus*

Figure 2 indicates increase in length of the fish in different groups, which showed a similar trend as observed in Figure 1 where Treatments B (LBTM) had the highest standard length (24.33±0.52 cm), followed by treatment A (MTH) 24.10± 0.52 cm, Treatment C (LGTM) (20.53±0.52 cm) while Treatment D had the lowest standard length gain (20.08± 0.52 cm), the standard length gain differ significantly at the end of twentieth week.

Mean percent survivability and sex ratio of masculinized *C. gariepinus* is presented in Table 2 Masculinized *C. gariepinus* of treatment MTH group had the lowest percentage survivability ((74.00% showing 37 fish out of 50 sexed fish with the highest sex ratio of 5:1 male to female fish) LBTM and LGTM group had higher percentage survivability of 92.00 and 80.00% respectively which were more than MTH group, and their sex ratio were 4:1 and 3:1 respectively, which were significantly higher ($P\leq 0.05$) than the sex ratio of the untreated control group (1:1). LBTM group of masculinized fish had the highest percentage survivability which was significantly higher than MTH group.

The survival rate were in accordance with the reports of Meyer et al. (2008) and Odin and Bolivar (2011) whom attributed higher survival of treatment with steroid and animal testes to the group that was treated with animal testes. The sex reversal of *Clarias gariepinus* reveals that treatments A, B and C (MTH, LBTM and LGTM) gave 83.34, 78.67 and 76.67% males, respectively. Previously, Popma and Green (1990), got 90% male Tilapia (*Oreochromis niloticus*) populations when they used 50 mg of

methyl testosterone hormone in 1 kg feed, Haylo and Pascual, (1991), also got successful results (70-80%) when they fed Tilapia fry with freeze-dried Ram testes meal, 97% male tilapia population when they treated fry with 10 mg/kg feed. Recently, Celik *et al.*, (2011), used methyl testosterone on Nile Tilapia fry at 0, 20, 30, 50 and 60 mg of mth/kg feed inclusion levels and got 57.1, 69.8, 69.4, 70.9, 86.1 and 93.7% males, respectively. Also, a good percentage of males up to (97%) was obtained in an indoor aquaria or outdoor hapas treatment of 17-alpha methyl testosterone at various doses of 15, 30, 45 and 60

mg/MT/kg feed (Phelps *et al.*, 2008). The synthetic androgen testosterone propionate was found to play a significant role in altering the sex of *C. gariepinus* into males among the four treatment tested, the dose of 30mg/kg feed was found to be most potent and resulted in 83.34% male fish, however had the lowest percentage survival which indicated that MT had effect on survival rate of sex reversed fish Sayed and Moneeb (2015).

Table 1: Proximate composition and testosterone level of the different treatment feeds

Parameter	Treatments (g/100g)			
	A	B	C	D
Moisture content	1.45 ± 0.01 ^b	2.00 ± 0.01 ^a	2.14 ± 0.01 ^a	1.45 ± 0.01 ^b
Ash content	9.90 ± 0.50 ^{ab}	9.13 ± 0.00 ^{ab}	8.88 ± 0.01 ^b	10.20 ± 1.15 ^a
Crude lipid	13.00 ± 0.00 ^b	15.61 ± 0.50 ^a	14.25 ± 0.40 ^{ab}	13.00 ± 0.00 ^b
Crude protein	59.12 ± 1.20 ^b	71.12 ± 2.20 ^a	69.31 ± 1.50 ^a	58.00 ± 1.10 ^b
NFE	16.03 ± 0.60 ^a	2.14 ± 0.02 ^c	4.40 ± 0.00 ^b	16.22 ± 0.50 ^a
CF	0.50 ± 0.00 ^a	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.50 ± 0.00 ^a
TL (ppm)	15.80 ^a	14.12 ^b	8.91 ^c	0.00 ^d

Means with different superscripts along row are significantly different ($P \leq 0.05$)
 TL= Testosterone Level; NFE= Nitrogen free extract; CF=Crude fibre; PPM=Parts Per Million

Table 2 Mean percent survivability and sex ratio of masculinized *C. gariepinus*

Treatments	No. of Fish Sexed	Survival	Male	Female	Total	Sex Ratio	
						♂	♀
MTH (30 mg/kg)	50	37 (74.00%) ^c	31 (83.34%) ^a	6 (16.22%) ^c	37	5:1	
LBTM (0.014 mg/kg)	50	46 (92.00%) ^a	36 (78.61%) ^b	10 (21.74%) ^b	46	4:1	
LGTM (0.0089 mg/k)	50	40 (80.00%) ^b	30 (76.67%) ^b	10 (25.00%) ^b	40	3:1	
Control (0.00 mg/kg)	50	40 (80.00%) ^b	19 (48.00%) ^c	21 (52.25%) ^a	40	1:1	

Means with different superscripts along column are significantly different ($P \leq 0.05$)

Table 3: Economics and cost benefit value of *Clarias gariepinus* masculinization using LBTM and LGTM

Variables	Treatments				
	MTH	LBTM	LGTM	Control	SEM
Feed Cost (₦)	29500.00 ^a	26000.00 ^b	26000.00 ^b	22000.00 ^c	±0.00
Total Revenue (₦)	96995.47 ^c	108196.13 ^a	98299.13 ^b	81867.42 ^d	±352.12
Total Cost (₦)	43930.00 ^a	43430.00 ^a	43430.00 ^a	33430.00 ^b	±0.00
Gross Profit (₦)	53065.47 ^b	64766.13 ^a	54869.13 ^b	48437.42 ^c	±217.22
Profit Index	3.29 ^c	4.16 ^a	3.78 ^b	3.72 ^b	±0.11
Incidence of Cost	26.82 ^a	20.00 ^c	22.62 ^b	24.44 ^b	±1.17
Benefit Cost ratio	2.21 ^c	2.49 ^a	2.26 ^{ab}	2.25 ^{ab}	±0.07

Means with different superscripts along column are significantly different ($P \leq 0.05$)

SEM= Standard Error Means; MTH= Methyl Testosterone Hormone; LBTM=Lyophilized Bull Testes Meal; LGTM= Lyophilized Goat Testes Meal; D= control (commercial feed only)

Table 3 shows the economics of masculinizing African catfish. The total cost of masculinization of *Clarias* using Animals testes was lower (₦43430) compared with that using synthetic hormone (MTH) (₦ 43930). The least production cost was obtained in the untreated group, LBTM masculinized *C. gariepinus* gave the highest gross profit (64766.13), profit index (4.16) and benefit cost ratio (2.49) while MTH masculinized fish had the lowest profit index (3.29) and

benefit cost ratio (2.21) of the control was the least (₦43,967.42). The untreated fish had the lowest gross profit value (₦48437.42), there was significant difference in the economics and cost benefit value across treatment groups ($P \leq 0.05$). Cost and return analyses computed showed a similar trend as reported by Odin and Bolivar (2011), where all natural androgen agents (lyophilized testes meal) gave higher return compared to synthetic agent (MTH), which was

as a result of the cost of MTH being higher than cost of lyophilized testes meal.

Conclusion and Recommendation

Masculinized *C. gariepinus* using LBTM gives better growth, sex ratio and economic returns, therefore should be adopted by fish farmers to boost growth of *C. gariepinus*.

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