CARCASS PROXIMATE COMPOSITION AND AMINO ACID PROFILE OF MASCULINIZED *Clarias gariepinus* (AFRICAN CATFISH)

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Abstract: Carcass proximate and amino acid composition of lyophilized bull and goat testes meal masculinized *Clarias gariepinus* was evaluated. Artificially bred *Clarias gariepinus* were masculinized, masculinization was carried out through feeding the fry with Lyophilized Bull Testes Meal (LBTM), Lyophilized Goat Testes Meal (LGTM) and commercial fish feed supplemented with Methyl Testosterone Hormone (MTH) as standard control for a period of four (4) weeks, at post juvenile stage, fish were fed commercial diet for five months. After five month of feeding, fish were anesthetized, gutted, freeze-dried and pulverized. Processed fish samples were stored in polythene bags for proximate and amino acid profile analyses. Proximate and amino acid composition of fish samples were determined following the standard methods of Association of Official Analytical Chemist (AOAC). Proximate and amino acid values of differently treated fish were subjected to one way Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) was used to separate mean were significant. LBTM masculinized fish had the highest muscle tissue crude protein and amino acid composition while non-masculinized fish (control group) had the lowest crude protein and amino acid composition, in conclusion masculinization increased carcass quality with respect to carcass crude protein and essential amino acids.

Keywords: *Clarias gariepinus*, masculinization, carcass quality, catfish

Introduction

Fish constitutes about 41% of the total animal protein intake by the average Nigerian hence there is great demand for fish in the country (Atanda, 2012). Nigeria with an estimate population of 160 million requires about 2.66 million metric tons of fish annually to satisfy the dietary requirement of her citizens. The total aggregate of domestic fish supply from all sources (capture and culture fisheries) is less than 0.7 million metric tons per annum (Atanda, 2012).

It has been established that high production cost is the limiting factor to the expansion of aquaculture industry in the world (Calbeg *et al*., 2000). The cost of feed is a major factor affecting fish production. Development of fish growth enhancing strategies is one of the major priorities for aquaculture research, not only to increase fish yield but also to reduce the risk of fish culture system failure (Offen *et al*., 2009). The African *Clarias gariepinus* male grow faster and reach a large final body size than the females (Turan, 2005), therefore it would be economically advantageous to produce all-male catfish (masculinize) populations.

Achieving this fit (masculinization) can be through administration of exogenous steroid can effectively work in controlling sexual development (Al-Ablani and Phelps, 2002). The synthetic steroids – 17 a-methyl testosterone is a male-specific hormone commonly used to induce sex reversal in teleost fish, Viveros *et al.* (2001) studied the effect of methyl testosterone on seminal vesicle development and semen release response of the African catfish (*Clarias gariepinus*). However, steroid treatment may have the disadvantages of being costly and health implications. It may lead to production of sterile populations and occasional paradoxical feminization with prolonged exposure at stages of gonadal development in the African catfish (Turan, 2005).

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 of goats and bulls may be a good alternative to androgens as agents of sex reversal in cultured fisheries. Haylo & Pascual (1991) sex reversed tilapia with diet prepared with Ram testes. Phelps *et al.* (1996) also conducted an efficacy experiment using Bull testes meal on sex inversion of tilapia, Odin and Bolivar (2000) Masculinized Tilapia with Lyophilized Bull, Boar and Caraboa testes meals, Turan & Cek (2007) masculinizes the African catfish (*Clarias gariepinus*) with a plant called tribulus (*Tribulus teristeris*). Fashina-Bambata & Somotu (2008) evaluated lyophilized goat testes meal on sex reversal of tilapia. Furthermore Liu *et al.* (2010) also conducted a sex reversal trial on the Southern catfish (*Silurus merdionalis*) and obtained a 50:50 ratio result. However, there is paucity of information on the protein and amino acid profile on this masculinized fishes which this study tries to address.

This study is geared towards evaluating carcass characteristics of lyophilized goat and bull testes meal masculinized *C. gariepinus*, considering *C. geriepinus* carcass proximate and amino acids profile.

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 1kg 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 a- 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-C_{18}). The column filters the sample down to 5 micro metre size (5 µm) and 250 mm by 4.6 mm at 25^{o}C. Automatic injection was performed through 20 µl loop on the automatic sampling unit then analytical detection was done using Ultraviolet (UV) light detector at 245 nm and ultrapure water at 45:55 v/v were used in the separation of analyte at running mobile phase at 1 ml/minute flow rate at 25^{o}C. This gives a good result; the sample was then optimized and cleaned using DSC-18 um at 6 ml/500 mg 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 h 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℃ for a period of 72 h 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 (0.2 mm copens) for 28 days. The fish were stocked in 12 plastic containers of 1000 liters capacity, each container contained 50 fish, and feeding of fish continued for five months with coppen

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 showed the 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±0.01 (diet A and D) and 2.14± 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±0.50, 9.13± 0.00, 8.88± 0.01 and 10.20±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± 0.00), significantly higher than diets A and D (13.00± 0.00 and 13.00± 0.00), while diet C closely the same with diet A. Crude protein of all the experimental diets were 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 1) 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 was slightly higher than lyophilized caraboar, bull and boar testes meal reported by Odin and Bolivar (2011) 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 that of 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.

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

Parameter (g/100g)	Treatments			
	A	B	C	D
Moisture	1.45±0.01^{b}	2.00±0.01^{a}	2.14 ±0.01^{a}	1.45±0.01^{b}
Ash	9.90 ±0.50^{ab}	9.13±0.00^{ab}	8.88 ±0.01^{b}	10.20±1.15^{a}
CL	13.00±0.00^{b}	15.61±0.50^{a}	14.25±0.40^{ab}	13.00±0.00^{b}
CP	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 (ppb)	15.80^{a}	14.12^{b}	8.91^{c}	0.00^{d}

^{abc}Means with different superscripts along row were significantly different ($P \leq 0.05$)

Crude lipid; Crude protein; TL= Testosterone Level; NFE=Nitrogen free extract; CF=Crude fibre; ppb=parts per billion

Table 2: Carcass proximate composition of masculinized *Clarias gariepinus* fed commercial diet

Contents (g/100g)	Initial	LBTM	LGTM	Control
Moisture	1.94± 0.01^{a}	1.42± 0.00^{c}	1.65±0.01^{ab}	1.44± 0.05^{b}
Ash	17.12±0.00^{a}	12.21± 0.00^{b}	10.27±0.00^{c}	10.97±0.01^{b}
CL	13.83± 0.00^{c}	14.67± 0.02^{a}	14.19±0.00^{b}	14.11± 0.00^{b}
CP	58.39±0.00^{d}	71.10± 0.03^{a}	69.71±0.03^{b}	68.67± 0.03^{c}
NFE	8.31±0.00^{a}	3.23± 0.00^{d}	3.83±0.00^{c}	4.38± 0.03^{b}
Crude fibre	0.41±0.00^{a}	0.37± 0.00^{b}	0.35± 0.00^{b}	0.43± 0.00^{a}

^{abc}Means with different superscript along row are significantly different (P<0.05); Crude lipid; Crude protein

The proximate composition of masculinized *C. gariepinus* fed commercial diet (Table 2), indicates moisture contents range from 1.42 ± 0.00% (LBTM fish carcass) to 1.92 ± 0.01% (initial fish carcass). The highest ash contents value was obtained from *C. gariepinus* masculinized with LBTM (12.21±0.00%), and LGTM fish carcass with the lowest ash contents (10.27±0.00%), the ash contents of fish carcass were significantly different (P<0.05). The percentage crude lipid contents of LBTM, LGTM and control group were 14.67±0.02, 14.21± 0.00 and 14.27±0.00%, respectively, carcasses quality of the experimental fish showed LBTM Fish carcass had the highest crude protein (CP) value (71.10± 0.03%), this was followed by LGTM with carcass CP values of 69.71± 0.03% and control had the lowest CP composition of 68.67± 0.00. Crude Protein content across the treatments were significantly different (P<0.05). Percentage nitrogen free extract NFE value ranged from 3.23 ± 0.00% (LBTM fish carcass) to 8.31±0.00% (initial fish carcass), crude fibre contents were 0.37 ± 0.00, 0.35 ± 0.00 and 0.43% for LBTM, LGTM and control fish carcass, respectively. Ajiboye *et al*. (2016) reported crude lipid, dry matter, ash and crude protein value of 7.24, 74.37, 6.92 and 66.55%, respectively for monosex tilapia, this indicated a lower crude protein value of fish compared to that of masculinized *C. gariepinus* in this study, however experimental fish carcass crude protein for all treatments were higher than initial fish carcass crude protein indicating that there was synthesis and increased muscle tissue protein production and masculinization had effect on protein and tissue protein production.

The amino acids profile of the masculinized fish carcasses after the experiment (Table 3), masculinized fish with LBTM diet had the highest amino acid profile for all individual amino acid, fish carcass of LBTM followed while the control (non-masculinization group) had the lowest amino acid profile, there was significant difference in the amino acid profile of fish carcasses(P<0.05). Valine was the amino acid with the highest value in all fish carcasses while histidine in LBTM (15.91 mg/g) and LGTM (11.66 mg/g) had the lowest value as well as leucine (8.25 mg/g) in the control group.

Table 3: Carcass essential amino acid profile of masculinized *Clarias gariepinus* fed commercial diet

Amino Acid (mg/g)	LBTM	LGTM	Control
Isoleucine	28.72^{a}	24.47^{b}	21.34^{c}
Leucine	18.53^{a}	14.28^{b}	8.25^{c}
Methionine	43.09^{a}	38.84^{b}	35.72^{c}
Phenylanine	24.34^{a}	20.09^{b}	16.97^{c}
Tryptophan	28.84^{a}	23.84^{b}	20.72^{c}
Valine	59.97^{a}	55.72^{b}	52.59^{c}
Lysine	20.33^{a}	16.08^{b}	12.95^{c}
Histidine	15.91^{a}	11.66^{b}	8.53^{c}
Threonine	23.11^{a}	18.01^{b}	14.44^{c}

^{abc}Means with different superscript along row are significantly different (P<0.05)

Amino acids which are the building blocks of protein, Campbell (2009), stated histidine help in the removal of heavy metal from the body, isoleucine help to increase endurance and help to heal and repair muscle tissue, leucine increases the production of growth hormone, lysine is needed for hormone production, methionine helps the body process and eliminate fat and phenylalanine is needed for normal functioning of the central nervous system. The amino acid profile of fish carcasses in this study were similar to that reported by Turan (2007) for stripped catfish carcasses. *Clarias gariepinus* masculinized with the LBTM and LGTM treatment showed better protein synthesis compared to control group which could be due the effect of masculinization.

Conclusion

Masculinized *Clarias gariepinus* has high crude protein and essential amino acid than non-masculinized *C. gariepinus.*

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References

Ajiboye OO Yakubu AF Simpa JO & Balogun SA 2016. Effect of Garlic-Supplemented Diets on Growth Response, Survival,Nutrient Utilization and Body Composition of Monosex *Tilapia zillii, World J. of Fish & Marine Sci.*, 8(2): 115-122.

Al-ablani SA & Phelps RP 2002. Paradoxes in exogenous androgen treatments of bluegill. *J. App. Ichth.*, 18: 61-64.

Atanda AN 2012. *Fish species Diversification in Aquaculture for the success of the Agriculture Transformation Agenda*: The role of Tilapia Production. In: Fisheries Society of Nigeria (FISON) Annual Public Lecture Abuja.

ALEP 2005. Inventory of feed producers in Nigeria. Published by Aquaculture and Inland Fisheries Project. Annex 1 of the National Special Programme for Food Security with the Agricultural Development Programme in all states and FCT Abuja, Nigeria. Pp1-8.

Al-ablani SA & Phelps RP 2002. Paradoxes in exogenous androgen treatment of bluegill. *J. App. Ichth.*, 18: 61-64.

AOAC 2000. *Official Methods of Analysis of Association of Official Analytical Chemists*, 17th Edition, A.O.A.C., Washington. DC, 21: 498-447.

Campbell MG 2009. The Nine Essential Amino Acids pp1-6, http://campbellmgold.com

Odin RY & Bolivar RB 2011. Masculinization of the Nile Tilapia (*Oreochromis niloticus*) using Lyophilized Testes from Caraboa (*Bubalus bubalis carabanensis* L.), Bull (*Bos indicus* L.) and Boar (*Sus domesticus* L.). Unpublished result of Aquafish Collaborative Research Support Programme. Freshwater Aquaculture Centre, Central Luzan state University Science City of Munoz, Nueva Ecija, Philiphines, p. 87.

Offem OB Gabriel UI & Ezekiel AO 2009. Effect of stocking size of the predatory African Catfish (*Heterobronchus longifilis* V.). On the growth performance of Nile Tilapia (*Oreochromis niloticus* T.). In Pond Culture. *Int. J. Fisheries and Aqua*, 1(3): 34-38.

Phelps RP 2006. Chapter 6: Hormone Manipulations of sex. Pp211-252 In: C.E. Lim and C.D Webster (eds). Tilapia: Biology. Culture, and Nutrition. New York. The Haworth Press, Inc. p678.

Phelps RP & Popma TJ 2000. *Sex reversal of tilapia*. In: B.A Costa-Peirce and J. E. Rakocy (eds). Tilapia Aquaculture in Americas. The World Aquaculture Society, Bato Rouge, Louisiana, United States, 2: 34-59.

Turan F 2005. *Effect of Natural (Adrosteredione and Red Clover) and Synthetic Hormones on sex reversal, Gonadal Development and Growth Performance of the African Catfish Clarias gariepinus* (Burchell, 1822) An unpublished Ph.D. Theses institute of Natural Sciences, Mustafa Kemal Univ. Hetay. In: Turan, F. and Cek, S. (2007). Masculinization of the African catfish (*Clarias gariepinus*) Treated with Gokshura (*Tribolium terrestris*). Mustafa University, Hatey.

Turan F & Cek S 2007. Masculinization of the African Catfish (*Clarias gariepinus*) Treated with Gokshura (*Tribulus teristris*). *The Israeli J. Aqua*, 59(4): 224-229.

Robyt JF & White BJ 1990. *Biochemical Techniques Theory and Practice*. Waveland: Prospect Heights.

Viveiros ATM Eding EH & Komen J 2001. Effect of 17-alpha-Methyl testosterone on seminal vesicle development and some release response in the African catfish (*Clarias gariepinus*). *Journal of Rep.*, 122: 817-827.