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Water quality and carcass proximate composition of *Clarias gariepinus* (Burchell 1822) juveniles fed blood meal graded diets

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Abstract

The water quality and carcass proximate composition of the juveniles of *Clarias gariepinus*, fed varying inclusion levels of blood meal in concrete ponds was investigated for a period of 270 days. Five Iso-nitrogenous (40% crude protein) diets were formulated with blood meal replacing fish meal diets at 0%, 25%, 50%, 75% and 100%. Three hundred (300) *Clarias gariepinus* juveniles of initial average weight and length of (11.45±0.25g and 9.00cm) were stocked at 20 fish per pond of five treatments diets in triplicate in a completely randomized design (CRD). The fishes were hand-fed at 8.00am and 4.00pm at 5% body weight daily. The data obtained from water quality parameters and carcass composition (initial and final) were analyzed using one way analysis of variance (ANOVA). The water samples analyzed for various physico-chemical parameters like temperature (26.79-27.13⁰c), pH (5.92-6.34), dissolve oxygen (5.82 - 6.05mg/l), transparency (15.84 – 16.70mg/l) and ammonia (0.13 – 0.24mg/l) and were within the recommended range for *Clarias gariepinus* culture. The body composition of the experimental fish carcasses showed that, there were significant difference in the nutrients contents of crude protein (68.00 - 69.50%), lipid (10.25–10.46%), NFE (13.33 – 13.88%), ash (8.07–8.29%) and lower fibre (8.51– 9.45%) contents at p>0.05 over the initial fish sample. This study concludes that, inclusion of blood meal at 25% as a replacement for fish meal in the diet of *Clarias gariepinus* does improve the nutritional composition of the whole fish produced and had no adverse effect on the water quality observed during culture.

Keywords: *Clarias gariepinus*, body composition, water quality parameters, blood meal

Introduction

Fish has always been reported as a cheap source of rich protein food for human consumption containing high quality vitamins (Vitamin A and D), minerals and other micro-nutrients (e.g. iron, zinc, calcium, magnesium and phosphorus. etc) that are very important, accounting for approximately 40% of Nigerians protein need ^[1, 2]. The African catfish, *Clarias gariepinus* (Burchell, 1822) belong to the family *Clariidae* and a major fish species for aquaculture in African countries ^[3]. It is cultured broadly in Nigeria under intensive or semi-intensive system because of its resistance to stress and disease ability, rapid growth rate, flesh tastiness, hardiness, good adaptability to environments, feed on large variety of agricultural by-products and highly commercial value ^[4, 3, 1]. Provision of a culture environment at its optimum level determines the success of the growth performance and survival rate of *Clarias gariepinus*. Fish ponds are man-made structure which is between 1cm² and 2ha (5acres or 20,000 m²) in area that holds water for months or years, thus creating an ecosystems that is often rich with diverse microbial life ^[5]. Growth and wellbeing of fishes are affected directly or indirectly by the quality of water in which they inhabit which include physical, biological and chemical factors as opined by ^[6]. Whereas water quality becomes poor, disease outbreak, poor growth, poor feed conversion efficiency and perhaps death may be inevitable. That is to say, fish must necessarily interact with water environment for the food chain to be complete ^[7, 8]. Hlavac *et al.* ^[9], reports that the physio-chemical constituents of water changes with changes in management techniques such as composition of stock and supplemented feed. The effect of such supplemented feeding on the water quality depends also on the constituents of the feed, its digestibility, palatability and technique employed during feeding ^[9]. Fish species of varying chemical composition require diverse nutritional make-up thus, in order to maximize carcass productivity, a good knowledge of fish composition is necessary.

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To achieve proximate composition of fish, crude protein, lipid, moisture, nitrogen free extract and ash content must be analyzed so as to establish the required nutritional content for the benefit of consumers [1, 10, 11]. Under culture conditions, it provides specific information and knowledge on its evaluation of fish health, body development, feed conversion efficiency and physiological state when influenced by feed composition, acceptability, utilization, culture environment, size, age and species (Shearer, 1994). According to [12, 13], individual fish carcass composition can also differ, however, the carcass of *Clarias gariepinus* is composed of ash (10.5%), nitrogen free excrete (10.20%), crude protein (52.20%), lipid (27.10%) moisture (75.00%). Blood meal, a common animal protein source with sustainable high protein contents of (85%), rich in lysine (7-9%), valine, leucine, phenylalanine and low in methionine [14] and heme-iron [15,14,1]. It has been recognized as one of the most competently good substitute for fish meal. Several studies have been reported on the potential use of blood meal as a replacement for fish meal component; in *Oreochromis niloticus* [20, 21] Gilthead sea bream [18], mudfish fingerlings [19] and *Clarias gariepinus* [20, 21] with vary degree of successes in their fish diets. In addition, blood meal according to Otubusin *et al.* [15] efficiently replaces fish meal of *Clarias gariepinus* with no adverse effects on growth rate, survival and feed conversion ratio. Although blood meal has been recognized as a rich protein source and good substitute of fishmeal with regards to growth, survival and yield of fish, its cumulative interaction in water can affect the general wellbeing of the fish. This study therefore evaluates the body composition and the corresponding water physio-chemical parameters of *Clarias gariepinus* juveniles fed graded levels of blood replaces diet during the period of culture.

Materials and methods

Study Area

The research was conducted at Amas fish farm located at Ezihe in Isiala-Mbano, Local Government Area of Imo State within Latitude 5° 40' 4" N and Longitude of 7° 12' 2" E. The fish ponds are made of concrete and designed in rectangular form with the main source of water at the farm being bore-hole water.

Experimental design

The experiment was designed as a completely randomized experiment (CRD) of five (5) treatments in triplicate. Fifteen concrete ponds of (4 m×4 m×1.5 m) were used for the experiment. Three hundred (300) juveniles of *Clarias gariepinus* of average standard length (11.45±0.25g) and length (9.21±0.91cm) were stocked at a density of 20

juveniles per pound and the experiment lasted for a period of 270days.

Experimental Fish

Three hundred (300) juveniles of *Clarias gariepinus* were purchased from kings' livestock enterprises (fish farm) in Owerri, Imo State. Fishes were conveyed to the site of the study in a fifty (50) liter plastic bucket filed with water to reduce stress. The initial length and initial body weight of the fishes were taken with a calibrated measuring board (to the nearest 0.1cm) and electronic weighing balance (Ohaus-30064419 scout pro, to the nearest 0.01gram) in order to establish the mean individual weight and length at the beginning of the experiment. The fishes were acclimatized for seven (7) days before they were distributed to various treatment ponds.

Experimental Diets

Commercial (vital) feed used as the control diet for the experiment was purchased from a commercial shop in Owerri, Imo State, Nigeria. Experimental feed was locally formulated using the Pearson square method for feed formulation with blood meal replacing fish meal component at 0%, 25%, 50%, 75% and 100% inclusion level respectively. Raw cow blood used was collected from the slaughter house, boiled for 30 minutes to reduce the water content and congeal the blood and then oven-dried at a temperature of 105°C. The dried blood was then finely grounded using Victorian hand grinding machine. Other locally available feed ingredients were fishmeal, soybean meal, maize, palm kernel cake, white maize, fish premix, salt, palm oil, starch and bone meal were purchased from a fish feed shop in Owerri, Imo State. All ingredients were bought in solid and dry form exception of palm oil. Prior to processing, the ingredients were milled individually to a fined powder, weighed according to the required percentage composition (table 1), manually mixed together and moistened thoroughly using the method described by [22]. The mixed diets were pelleted using electrical pelletizing machine set at 2mm, 4mm and 6mm, sun-dried for 4days to remove moisture, packaged into separate bags, labeled and stored in a cool dry place. The formulated experimental feed (table 2) were analyzed in triplicate for the proximate composition to confirm the formulation using standard method of Association of Official Analytical Chemists [23] at Dry and Wet Laboratory of the Department of Fisheries and Aquaculture, Federal University of Technology Owerri, Imo State. The proximate composition of the commercial feed used was that of the manufacturing company.

Table 1: Percentage composition of feed ingredients (g/100g)

Ingredients	Control (0%)	T1 (25% BM)	T2 (50% BM)	T3 (75% BM)	T4 (100% BM)
Fish meal	24.0	18.0	12.0	6.0	----
Blood meal	----	6.0	12.0	18.0	24.0
S.M.B	24.0	24.0	24.0	24.0	24.0
Maize flour	40.0	40.0	40.0	40.0	40.0
Bone meal	2.0	2.0	2.0	2.0	2.0
P.K.C	5.0	5.0	5.0	5.0	5.0
Starch (binder)	2.0	2.0	2.0	2.0	2.0
Salt	0.5	0.5	0.5	0.5	0.5
Vit/min premix	1.5	1.5	1.5	1.5	1.5
Palm oil	1.0	1.0	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0	100.0

Fish Feeding and Tank Management

The fishes were hand-fed two times daily between 8.00am and 4.00pm at 5% body weight as described by [24]. Subsequent weight and total length measurements were taken every two weeks to determine the fish growth rate using an Ohaus. The ration was adjusted when new weights of the juveniles for the various experimental ponds were determined. Leftover feeds and faeces were siphoned out every two days and replaced with fresh water as described by [25] and [26]. Water quality parameters were monitored weekly using digital multi-parameter water meter. The amount of feed consumed, survival and mortality of fish in each treatment pond were monitored by counting the fish in the pond and recorded accordingly.

Laboratory Analysis

Proximate composition analysis of experimental diets and experimental fish

Proximate composition of experimental diets and fish (initial and final) were carried out according to the procedures of the Association of Analytical Chemist (AOAC, 2005) [23] at Laboratory of Fisheries and Aquaculture, Federal University of Technology, Owerri, Imo State. The moisture and dry matter contents were determined by oven-drying the sample for 4hrs at 105°C until a constant weight was obtained. Crude protein (N×6.25) was determined using the routine semi-Kjeldahl method. Crude fat was determined by Soxhlet extraction method using petroleum ether extractor whereas crude fiber was quantified by acid digestion followed by ashing the dry residue at 550°C in a muffle furnace for 4 hours. Nitrogen free extracts content was determined by subtracting the percentage of moisture, crude protein, ash and fat from 100.

Water Quality Analysis

Sample collection

Water samples were collected aseptically from different concrete ponds per replicate using sterile plastic bottles (1000

ml), tightly sealed and labeled. The water samples were transported to the Dry and wet laboratory of the Department of fisheries and Aquaculture, Federal University of Technology Owerri, Imo State.

Physico-chemical analysis of pond water samples

Water samples were analyzed for physical and chemical quality parameters as described by APHA *et al.*, (2005) [27]. Parameter such as temperature was measured using a mercury filled Celsius thermometer [28], pH and dissolve oxygen measured using digital multipurpose meter (Hanna H12210). Water transparency was measured using secchi disk according to Anyanwale *et al.* [29], turbidity determined using Nephelometric method using turbid meter (Hanna H188703) and alkalinity, water hardness, phosphate, nitrate and ammonia were determined in the laboratory using standard laboratory techniques [27].

Statistical analysis

Data obtained were subjected to One-way Analysis of Variance (ANOVA) as described by [30]. Significant differences between mean were determined using Duncan's multiple range tests ($p < 0.05$) with the aid of Statistical Package for Social Sciences (SPSS) version 20.

Results

The proximate composition of the experimental diets (table 2) shows that Crude protein was highest in control diet (40.00%) followed closely by T2 (38.84) and lowest in diet 3 (38.25%). Crude fiber in T3 was 5.33% which superseded that of the control (5.33%) and recorded its lowest value in T2 (5.14%). Ash content in T1 (8.17%) was highest, T2 (7.85%), T4 (7.62%), with only T3 (7.40%) being greater than control (7.50%). Lipid, Dry matter and caloric value recorded their highest value in the control diet at 12.20%, 93.16% and 10.44% respectively. NFE was highest in T4 (29.35%) but had T1 (27.08%) and T3 (27.12%) being less than the control diet (27.32%).

Table 2: Proximate Composition of the Experimental Diets.

Parameters	Control** (0%)	T1 (25%)	T2 (50%)	T3 (75%)	T4 (100%)
Crude Protein (%)	40.00	38.29	38.84	38.25	38.42
Crude Fiber (%)	5.33	5.20	5.14	5.53	5.19
Lipid (%)	12.20	12.09	11.34	12.10	11.28
Ash (%)	7.50	8.17	7.85	7.40	7.62
Moisture (%)	12.17	13.19	11.00	11.54	11.69
NFE (%)	27.32	27.08	27.64	27.12	29.35
Dry Matter (%)	93.16	91.33	91.09	91.13	91.38
Caloric Value (kcal/g)	10.44	10.22	10.34	10.17	10.27

**Manufacturer's nutrient specification

T1- 4 = Treatments 1-4

The proximate analysis of experimental fish before and after the study in (table 3) showed that there was an increase in fish carcass in respect to crude protein, lipid, ash, N.F.E, moisture content and dry matter, however, fibre content was reduced from 10.4±0.35 for the initial analysis to as low as 8.51 ± 0.36 in T2 at the end of experiment. Control diet (69.50%) recorded the highest value and diet T2 (67.50%) the least value in the carcass protein content. The carcass lipid content was high in control diet (10.46%) and low in T1 (10.25%). Ash content recorded increasing values with the increasing percentage substitution of blood meal (control 8.11±0.03 and

T4 8.29±0.25). On the other hand, the moisture content reduced as blood meal substitution increased but slightly increased in T4 (53.19± 0.08). same was recorded for N.F.E carcass content which increased as the blood meal content increased, the highest being that of the control diet (13.88±0.56) and lowest in diet T4 (13.33±0.19). The carcass dry matter of fish fed diet T1 (91.64%) had values than the control (91.52±0.36) but progressively reduced in T2, T3 and T4 (91.12±0.08, 91.13±0.13 and 89.32±0.24) respectively. The whole body carcass showed a significant difference at $p < 0.05$ in values except for lipid which was not significant.

Table 3: Percentage Body Composition of Experimental fish fed blood meal graded diets.

Parameters	Initial	Final				
		Control (0%)	T1 (25%)	T2 (50%)	T3 (75%)	T4 (100%)
C. P.	54.30±0.18	69.50±0.06	69.08±0.11	67.50±0.40	68.30±0.19	68.10±0.32
Lipid	9.22±0.07	10.46±0.40	10.25±0.13	10.35±0.30	10.26±0.06 ^a	10.43±0.20
Ash	7.27±0.18	8.11±0.03	8.15±0.13	8.07±0.07	8.23±0.06	8.29±0.25
Crude fibre	10.40±0.35	9.16±0.10	9.30±0.12	8.51±0.36	9.45±0.27	9.24±0.04
NFE	10.26±0.14	13.88±0.56	13.59±0.45	13.40±0.24	13.43±0.23	13.33±0.19
M.C	49.22±0.03	54.19±0.16	53.41±0.33	50.20±0.17	50.20±0.16	53.19±0.08
Dry matter	80.18±0.11	91.52±0.36	91.64±0.55	91.12±0.08	91.13±0.13	89.32±0.24

Mean values without superscript are significantly different ($p < 0.05$). NFE= Nitrogen Free Extract, M.C = moisture content, C.P = Crude protein.

Table 4 shows the water quality parameter results obtained during the experiment. There was no significant difference ($p > 0.05$) in the recorded water temperature, Dissolved oxygen, pH, turbidity, nitrate, alkalinity and water transparency between the treatments. Temperature ranged from 26.79±0.89°C – 27.13±0.87°C, dissolved oxygen ranged from 5.81±1.09mg/l – 6.26±0.81mg/l, pH ranged from 5.92±0.79 – 6.53±0.77, turbidity ranged from 1.34±1.24 NTU – 1.94±1.67NTU, alkalinity ranged from 21.20±1.29mg/l – 22.08±1.8mg/l, nitrate ranged from 0.49±0.51mg/l –

0.71±0.88 and water transparency ranged from 15.85±1.37cm – 16.70±1.87cm. Significant difference was obtained in water hardness, phosphate and ammonia-nitrogen with water hardness ranging from 20.74±0.88mg/l – 28.07±2.41mg/l; phosphate, from 0.41±0.48mg/l – 1.13±1.18mg/l and ammonia-nitrogen ranging from 0.09±0.06mg/l – 0.24±0.17mg/l. Analysis revealed that these obtained values were quite within the recommended range for *Clarias gariepinus* in concrete pond (Boyd, 1979) [28].

Table 4: Water Quality Parameters taken during feeding experiment of *Clarias gariepinus*.

parameters	Control (0%)	T1 (25%)	T2 (50%)	T3 (75%)	T4 (100%)
DO (mg/l)	5.82±1.08	5.95±1.11	5.81±1.09	6.26±0.81	6.05±0.89
pH	6.34±0.73	6.53±0.77	6.30±0.87	6.26±0.81	5.92±0.79
T(°C)	27.05±0.89	27.13±0.87	26.85±0.79	26.79±0.89	26.80±0.90
NH ₃ N (mg/l)	0.24±0.17 ^a	0.13±0.11 ^b	0.21±0.08	0.09±0.08 ^c	0.09±0.06 ^d
PO ₄ (mg/l)	0.41±0.48	0.93±1.02	1.08±0.98	0.62±0.72 ^a	1.13±1.18
Alkalinity	21.40±1.41	21.57±1.44	22.08±1.08	21.97±1.45	21.20±1.29
Hardness	28.07±2.41	23.16±3.06 ^a	20.81±0.89 ^b	21.01±0.83 ^c	20.74±0.88 ^d
Turbidity	1.60±1.36	1.74±1.65	1.94±1.67	1.76±1.37	1.34±1.24
Transparency	16.54±2.25	16.18±2.25	16.66±2.05	16.70±1.89	15.84±1.37
Nitrate	0.71±0.88	0.63±0.71	0.52±0.54	0.52±0.54	0.49±0.51

Mean values with different superscript are significantly different ($p < 0.05$). DO=Dissolved Oxygen, NH₃N= ammonia-nitrogen, T= temperature and PO₄= phosphate.

Discussion

The ideal knowledge of the proximate composition of fish feeds (protein, fat, ash, NFE, fibre and moisture) contents are very important because it gives a reflection on the dietary and health importance of these nutrients in fish and also to final consumers which is man [31, 2]. Fish feeds components play a vital role in growth response, body development, metabolisms, repairing worn-out tissues and survival of fish. Proximate composition of the experimental diets indicated that the obtained crude protein values (38.48% - 40.00%) in this study were within the protein requirement (30%-40%) as recommended for growth performance in *Clarias gariepinus* as reported by [32]. This also agrees with the study of Otunbusin *et al.* [15]. The knowledge of fish proximate composition provides a basis for the assessment of the nutrient quality that is made available to the consumers. The body composition of the experimental fish after the experiment revealed increased carcass crude protein, lipid, NFE, ash, moisture and lower fibre contents than the initial fish sample. These results shows that inclusion of blood meal in the diets significantly affect the carcass of fish in a positive way and agrees with [33], who Reported that the whole proximate composition of *Clarias gariepinus* fed with blood meal substitute, at the end of experiment showed a change in value when compared to those of the control values. The proximate composition of the fish carcass shows an increase

in the value of crude protein and lipid over the initial fish samples. There was a reduction in its values as feed were substituted with blood meal. This agrees with the reports of [34, 35, 36] and could be attributed to the crude protein content of the experimental diets which was readily converted and protein utilized increasing tissue production and real growth. The increase in fish carcass lipid indicates an enhanced production of lipids in fish without producing an excessive fatty carcass which shows a significant difference ($p < 0.05$) among the treatments. Fafioye *et al.* [36] observed similar increase in lipid content of *Clarias gariepinus* fed soya-based diets. Same was also observed in gilthead bream fingerlings [35] where carcass lipid was associated with increased metabolism efficiency. The significant difference recorded in the percentage ash and fiber content obtained from the carcass of the fishes fed experimental diets may be attributed to the adequate levels of ash and fiber in the blood meal diet which was transferred into the body of fish for optimal growth. The values obtained were within the range reported by [37], who opined that ash and fibre content of not more than 8-12% is needed for optimal fish growth. The fish moisture content were significantly different at $p < 0.05$ compared to the initial value, but were lower to the values (72%-75%) reported by [38, 39]. According to Suzanne [40], increasing moisture content observed in fish carcass is considered beneficial because it helps in the stabilization of fish during locomotion. Dry matter and nitrogen free extract shows a significantly different

($p < 0.05$) in fish carcass at the end of the experiment when compared with those in the initial values. However, several studies on knowledge of the chemical composition of *Clarias gariepinus* [41, 42, 36] helps to ascertain the nutritive value, development and physiological state of fish when introduced to new formulated feeds and culture environment thereby promoting suitable, tasty and edible good fish when consumed. Water is an essential habitat of fish and its quality component must be present for optimum growth and survival of fish in aquaculture sector [43]. Fish completely depends on water for feeding, respiration, excretion, growth and reproduction, etc. Thus, a successful aquaculture business is dependent on good water quality management plus fish health. The water quality parameters measured during the study period were within the ranges recommended for the culture of *Clarias gariepinus* [44, 45, 48]. This quality parameters were not affected by the diet forms as the blood meal formulated does not significantly deteriorate the water as the diets were formulated correctly and the fish were fed optimally. The physico-chemical parameter results obtained agrees with the reports of [28, 43, 45, 46, 47, 48, 49]. Water quality directly affects the fish health and survival. The continuous water exchange and regulated feeding rate contributed to the optimal growth of the catfish as observed by [51, 52].

Conclusion

This study concluded, that the inclusion of blood meal as a substitute for fish meal in the diet of *Clarias gariepinus* have increased effect on the body composition of the fish produced and had no adverse effect on water quality parameters. Blood meal is an animal waste with a nutrient profile close to that of fish thus, good substitute for fish meal which supports the fish farming sector. Its usage is hoped to reduce the cost of aqua feed production by utilizing the waste by-product for profit and sustainable aquaculture. Also water quality should be closely monitored to serve as guide for managing a pond to avoid adverse conditions that could affect growth and survival of catfish.

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