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Growth Performance, Feed Utilization and Body Composition of *Clarias gariepinus* (Burchell 1822) Fed Marine Fish Viscera-based-diet in Earthen Ponds

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Abstract

A 90-days experiment was conducted to study the effect of replacement of fishmeal (FM) with marine fish viscera (MFV) meal on growth performance, body composition and production of *Clarias gariepinus* fingerlings (mean weight 11.3 ± 0.1 g). Diets were three isonitrogenous (43% crude protein) and isoenergetic (20 KJ/g) diets containing 0% (D0), 30% (D30) and 50% (D50) of MFV, as FM substitute. Diet D0, without MFV, acted as a control. All these diets were compared to the commercial diet coppens developed for *C. gariepinus*. No significantly differences were found in final weight (range: 220.94-234.1 g), weight gain (range: 1937.2-1971.7%), specific growth rate (range: 3.30-3.37%/day), protein efficiency ratio (range: 1.93-2.09) and annual production (range: 378.3-415.0 kg/are/year) of fish fed coppens diet, D0 and D30 ($p>0.05$). Fish fed D50 showed significantly lower growth and feed utilization performances ($p<0.05$). Moisture and crude protein were similar among dietary treatments ($p>0.05$). Lipid deposition in fish significantly increased with MFV level in diets, whereas ash content decreased ($p<0.05$).

The study indicates that MFV meal can be used up to 30% in formulation fish feed for promotion of *Clarias gariepinus* rearing in rural areas.

Keywords: *Clarias gariepinus*; Fishmeal replacement; Marine fish viscera; Growth; Earthen ponds

Introduction

Fishmeal is the main ingredient for most fish diets because of its high protein content, balanced amino acid profile, high essential fatty acids content, minerals and vitamins [1-6]. As a consequence of rapid growth of aquaculture, fish meal prices have increased significantly in the past few years and are likely to increase further with continued growth in demand [7-10]. Considering the global increasing of human population, feeding FM to farmed fish on any significant scale is neither profitable nor sustainable, especially in developing countries where the use of FM in fish feed is often economically prohibitive [11,12]. Thus, studies on the use of other efficient and cheaper sources of protein as substitutes for fish meal are necessary for aquaculture development and durability [6,13].

African catfish *C. gariepinus* is a globally popular aquaculture species largely distributed throughout Africa and Asia [2,14-18]. It is widely cultured in freshwater ponds because of their easiness in reproduction, high growth rate, tolerance to high densities culture conditions, resistance to diseases, excellent flesh quality and ability to accept a wide variety of feed [15-17,19]. The technics culture for the full life cycle of African catfish has been well-established and the global production of this species has been increased from 11.8 tons in 2000 to 517.4 tons in 2010 [20]. However, its intensive culture is quite limited because of the high operational cost due to the high protein commercial diets which increased feed cost [19,21]. The economically feasible catfish farming can be achieved when it is based on cost-effective feed compound of locally available agricultural by-products [17,22,23]. Many alternatives resources such as feather meal [19], meat and bone meal, hydrolyzed feather meal, fleshings-meal and blood meal [24,25], dried fermented fish by-product silage [6], poultry silage [26], shrimp head waste meal [27,28], poultry by-product meal [2,29], skate meal and sablefish viscera meal [30] have been tried to replace fish meal either partially or fully, but even these meals of various animal sources are not sufficient to meet the growing demands of fish raising industry.

Appropriate use of local protein by-products could reduce feed costs and enhance environment and economic sustainability [30]. Marine fish viscera are non-edible parts produced as by-product in large quantities in Benin by fish processing industries. These wastes are being dumped in close vicinity to market and at sea. It is challenged to recycle these wastes into acceptable source of animal protein in diets for fish [31,32]. Marine fish viscera have likely similar nutritional qualities as the fish meals currently used in aqua feeds [33,34]. It includes significant quantities of lipids with long chain, highly digestible, well-balanced proteins and highly unsaturated (n-3) fatty acids [30,35]. Several works reported isolation and identification of polyunsaturated fatty acids especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), enzymes and other bioactive compounds from marine fish viscera [36-42]. Indeed, EPA and DHA are important omega-3 polyunsaturated fatty acids of which human body needed but cannot produce [42]. They were confirmed to benefit the functions of various systems in human body, including cardiovascular health, brain health, eyesight health etc. [43-51]. The purposes of the present study were to feed *C. gariepinus* with marine fish viscera and to evaluate its efficacy in terms of growth performance, feed efficiency and change in whole-body carcass composition.

Materials and Methods

Fish viscera meal

Marine fish viscera was rendered from commercial fish processing industry in market place and stored frozen (-20°C). After, it was slightly heated and dried in oven at 55°C for 48 h. The dried product was grounded and meal was stored in a refrigerator in plastic bag until used.

Experimental ingredients and diets

FM used in this study was Sardinella aurita meal. Slaughter house blood was collected from Calavi town immediately after the slaughtering of oxen. The blood was allowed to clot and only the clotted portion was collected and immediately brought to the laboratory. At the laboratory, blood was heated and sun-dried for three days. Maize bran (Zea mays), palm oil and soybean oilcake (Glycine max) were purchased at the local market, the amount of the latter being kept at 10-15%, so as to minimize the effects of its anti-nutritional factors. Dried viscera, sun-dried blood, maize bran and soybean oilcake were grounded and separately stored in refrigerator at +4°C until used. Three isoproteic (43% crude protein) and isoenergetic (20 KJ/g) experimental diets (Tables 1 and 2) were formulated to meet the protein and energy requirements of the juvenile catfish. Diet D0 contained FM as the main animal protein and was considered as the control diet. In diets D30 and D50, MFV meal was incorporated to replace partially and completely the FM. All diets were compared with coppens diet in order to validate our experimental facilities and diets. The ingredients and diets were analyzed for the proximate composition using standard methods given in Millamena [52] and the results are presented in Tables 1 and 2, respectively.

All ingredients were grounded in grinding mill to desired particle size, weighed and mixed thoroughly in a food mixer for 30 min. The hot water (about 30% of dry weight diet) was progressively added to one kilogram of diet formulated and blended. The resulting dough was cut into paste and sun-dried for about three days at 32-35°C. After drying, the diets were broken into small particles (mm) and preserved in refrigerator (+4°C) until used. The formulation of the experimental diets is given in Table 2.

Ingredients	Dry matter	Crude protein	Crude lipid	Ash
Fish meal	92	66	7.88	15.77
Bood meal	90.9	71.9	1.7	6.4
Maize bran	91.4	6.2	3.1	1.4
Soybean oilcake	94.8	30	13.2	3.7
Marine Fish viscera	27	38.8	39	7

Table 1: Proximate composition (expressed as percent dry matter) of feeds ingredients.

Fish rearing, experimental design and feeding

The experiment was carried out during 90 days in wetland area at Louho, Porto-Novo, Benin. One-thousand and nine hundred (1900) *C. gariepinus* fingerlings (average weight 11.3 ± 0.1 g) were obtained from the Tonon fish farming foundation located at Calavi and were transported to the experimental station.

Ingredients (%)	Diets			
	Coppens*	D0	D30	D50
Fish meal		30	15	0
Blood meal		23	23	23
Maize meal		30	20	10
Soybean oilcake		15	10	15
Fish viscera meal		0	30	50
Palm oil		2	2	2
Proximate composition (%.MS)				
Dry matter	89.4	90	88.4	90.3
Crude protein	43	43	43.3	43.2
Lipid	13	10.8	12.3	12.9
Ash	9.9	13.1	12.6	12.7
Carbohydrate	34.1	31	31.8	31.2
Gross energy (KJ g-1)	21.2	20.2	20.6	20.7

Table 2: Formulation and proximate biochemical composition of experimental diets. D0, diet containing fish meal; D30, diet containing 30% fish viscera meal; D50, diet with 50% fish viscera meal.

Fish were randomly stocked into twelve earthen ponds (10 m×3 m×1 m) at a density of 5 fish m^{-2} (150 fish per pond). They were acclimated to experimental conditions for three days in ponds during which time all fish were fed a mixture of two experimental diets twice daily. At the start of the experiment, the acclimated fish were deprived of feed for 24 h. Ponds were grouped into four triplicate and each was randomly assigned an experimental diet. All ponds were filled naturally from water table. During the feeding trial, fish were hand-fed to apparent satiation at 09:00 and 17:00 hours daily. Care was taken to stop feeding as soon as the fish stopped eating. At each fortnight, 40% of fish in each pond were sampled out with a seine net (12.7 mm mesh size) and weighed [53,54].

Fortnightly, temperature, dissolved oxygen, pH, conductivity and total dissolved solid (TDS) were measured at a deep of 10 cm using multiparameter HANNA HI-9828. Water transparency was measured with Secchi disk. Nutrients such as nitrite and ammonium were determined by cadmium reduction and phenate methods respectively. Zooplankton abundance was also carried out.

Biochemical Analysis

One-hundred randomly chosen fish were sampled from the initial population to determine initial carcass composition. For final carcass composition analysis, twenty fish were randomly selected from each pond. Samples were analyzed according to standard method [52] for dry matter and total ash. Dry matter was evaluated from weight loss after drying in an oven at 105°C for 24 h. Crude protein was determined by the Kjeldhal technic (protein=N×6.25). Total lipid in fish carcass was extracted by chloroform-methanol method [55]. Ash value was evaluated from weight loss after incineration of samples in a muffle furnace for 24 h at 550°C. Total carbohydrates was estimated by subtracting crude protein, lipid and ash values from 100. Gross energy was then calculated on the basis of 23.7 KJ/$g_{protein}$, 39.5 KJ/g_{lipids}, 17.2 KJ/$g_{carbohydrate}$ [24].

Growth parameters

Growth performance, survival and feed utilization were evaluated as below : Survival (S,%)=100×(final count) / (initial count), weight gain (WG,%)=100×[(wf - wi)/wi], specific growth rate (SGR,% day^{-1})=100×[ln (wf) - ln (wi)]t^{-1}, Feed conversion ratio (FCR)=TFI (FB - IB)$^{-1}$, Protein efficiency ratio (PER)=(FB - IB) / DPI, Yield (Y, kg/are)=(FB - IB)/S, Production (P, kg/are/year)=([FB - IB) S^{-1}]×365) t^{-1} ;

where wi and wf=initial and final mean body mass (g); t is the duration of experiment (days); FB is the final biomass per pond (g); IB, the initial biomass per pond (g); TFI, the total food intake (g); DPI the dietary protein intake; S, pond superficies.

Statistical analysis

All data were subjected to a one-way analysis of variance (ANOVA) to test the effect of replacement of fishmeal. Differences between means were determined by Student-Newman-Keuls post hoc tests and were considered to be significant when P-values were <0.05. Before analysis, homogeneity of variance was checked using the Hartley statistical test [56,57] after log-transforming. All analyses were done using the statistical package SPSS version 22.0 for windows (SPSS, Chicago, Illinois, USA).

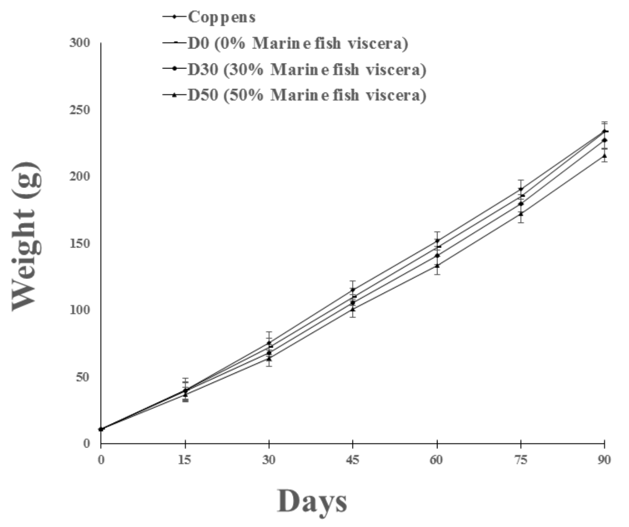

Figure 1: Growth of *Clarias gariepinus* fed marine fish viscera-diets in earthen ponds.

Parameters	Diets			
	Coppens	D0	D30	D50
1.Water quality				
Transparency (cm)	15.6 ± 2.8	16.4 ± 2.4	17.2 ± 1.5	16.8 ± 1.3
Temperature (°C)	29.7 ± 1.8	29.3 ± 1.5	29.6 ± 1.6	28.9 ± 1.3
pH	6.0 ± 0.5	5.7 ± 0.2	5.9 ± 0.4	6.2 ± 0.4
Dissolved oxygen (mg l^{-1})	4.3 ± 2.5	4.2 ± 1.7	4.2 ± 2.7	3.8 ± 2.3
Conductivity (µS cm^{-1})	115.6 ± 12.3	110.1 ± 15.3	109.4 ± 15.2	112.8 ± 14.0
Nitrite (mg l^{-1})	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Ammonium (mg l^{-1})	0.05 ± 0.0	0.06 ± 0.01	0.05 ± 0.02	0.04 ± 0.01
2. Zooplankton (number l^{-1})	1531.3 ± 26.3^a	600 ± 27.5^d	1038.8 ± 608.6^b	837.5 ± 705^c
(%) Copepods	49	56.3	49.6	44.5
(%) Rotifers	35.7	39	36.6	39.6
(%) Cladocerans	15.4	4.8	13.8	16
Means values in the same row having different superscript are significantly different (p<0.05). D0, diet containing fish meal; D30, diet containing 30% fish viscera meal; D50, diet with 50% fish viscera meal.				

Table 3: Values (Means ± SD) of water quality parameters and zooplankton density (number/l) in different treatments during 90-days trial. D0, diet containing fish meal; D30, diet containing 30% fish viscera meal; D50, diet with 50% fish viscera meal.

Results

Water quality characteristics in all ponds during the 90-days trial are summarized in Table 3. The water transparency ranged from 15.2 ± 2.9 to 16.4 ± 2.7 cm, temperature from 28.91 ± 1.31 to 29.73 ± 1.76°C, pH from 5.76 ± 0.23 to 6.17 ± 0.36, dissolved oxygen from 3.82 ± 2.32 to 4.26 ± 2.48 mg l^{-1}, conductivity from 109.4 ± 15.2 to 115.6 ± 12.3 (µS cm^{-1}), total dissolved solid from 50.28 ± 8.5 to 52.00 ± 6.6 ppm, ammonium 0.04 ± 0.01 to 0.06 ± 0.01 mg l^{-1}, nitrite 0.01 mg l^{-1}. There were no significant differences between all these parameters measured during the experimental period (p<0.05).

Parameters	Diets			
	Coppens	D0	D30	D50
Initial weight (g)	11.3 ± 0.1	11.3 ± 0.1	11.3 ± 0.1	11.3 ± 0.1
Survival (%)	90.0 ± 2.9	91.0 ± 2.5	91.0 ± 2.5	93.0 ± 0.6
Final weight (g)	234.1 ± 5.7^{a}	233.3 ± 4.5^{a}	230.2 ± 4.1^{a}	220.9 ± 3.1^{b}
Condition factor	0.96 ± 0.11^{a}	0.95 ± 0.09^{a}	0.97 ± 0.12^{a}	0.91 ± 0.08^{b}
Feed intake (g fish^{-1})	248.3 ± 3.2^{b}	247.2 ± 3.6^{b}	256.9 ± 3.4^{a}	247.9 ± 3.4^{b}
SGR (% days^{-1})	3.37 ± 0.03^{a}	3.36 ± 0.02^{a}	3.35 ± 0.02^{a}	3.30 ± 0.02^{b}
WG (%)	1971.7 ± 50.4^{a}	1964.6 ± 39.8^{a}	1937.2 ± 36.3^{a}	1852.8 ± 27.4^{b}
FCR	1.12 ± 0.03^{b}	1.11 ± 0.02^{b}	1.13 ± 0.02^{b}	1.20 ± 0.03^{a}
PER	2.08 ± 0.04^{a}	2.09 ± 0.03^{a}	2.05 ± 0.02^{a}	1.93 ± 0.03^{b}
Yield (kg are^{-1})	100.3 ± 2.6^{a}	101.0 ± 2.0^{a}	102.3 ± 2.8^{a}	94.3 ± 2.4^{b}
Production (kg are-1 year^{-1})	406.6 ± 10.4^{a}	409.7 ± 8.3^{a}	415.0 ± 7.8^{a}	378.3 ± 5.6^{b}

Values are means ± SD of three replications. Values in the same row having different superscript are significantly different (p<0.05). D0, diet containing fish meal; D30, diet containing 30% fish viscera meal; D50, diet with 50% fish viscera meal.

Table 4: Means (values ± S.D.) of growth parameters and annual production of *C. gariepinus* fed marine fish viscera-diets in earthen ponds for 90 days. D0, diet containing fish meal; D30, diet containing 30% fish viscera meal; D50, diet with 50% fish viscera meal.

Diets	Moisture	Crude Protein	Crude Lipid	Ash
Initial	10.79 ± 0.23	60.48 ± 0.88	15.13 ± 0.1	13.68 ± 0.23
Coppens	10.77 ± 0.09	61.39 ± 1.53	13.19 ± 0.07^{c}	14.53 ± 0.50^{a}
D0	10.55 ± 0.28	61.42 ± 0.98	14.32 ± 0.78b^{c}	14.53 ± 0.10^{a}
D30	10.32 ± 0.15	60.53 ± 0.43	17.48 ± 0.23^{a}	13.63 ± 0.32^{b}
D50	10.43 ± 0.40	59.56 ± 0.24	18.65 ± 0.57^{a}	13.42 ± 0.47b

Values with different superscript are significantly different (p<0.05). D0, diet containing fish meal; D30, diet containing 30% fish viscera meal; D50, diet with 50% fish viscera meal.

Table 5: Proximate composition (%) of whole body of *Clarias gariepinus* fed the experimental diets. D0, diet containing fish meal; D30, diet containing 30% fish viscera meal; D50, diet with 50% fish viscera meal.

There were significant differences between absolute density and relative abundance of zooplankton of experimental ponds (p<0.05). The high absolute density value was obtained in ponds receiving coppens diets (1531 ± 26 individual l^{-1}) and the lowest value was observed in ponds receiving D0 (600 ± 27 individual l^{-1}). Furthermore, relative abundances of rotifers and copepods were of highest values in all ponds (Table 3).

Figure 1 presenting changes in fortnight mean weight of experimental fish showed growth overtime. Growth performances, survival rate and feed utilization parameters value were shown in Table 4. Survival rate was similar among dietary treatments. There were no significant differences (p>0.05) in final weight (220.9-234.1 g), weight gain (1937.2-1971.7%), specific growth rate (3.30%/day-3.37%/day), feed conversion ratio (1.11-1.20), protein efficiency ratio (1.93-2.09) and annual production (378.3-415.0 kg/are/year) of fish fed with coppens, D0 and D30, the best performances being obtained with fish fed coppens diet. Significant differences (p<0.05) were found in feed intake. Fish consumed D30 (256.93 ± 3.41 g/fish) much more than those diets coppens (248.30 ± 3.19 g/fish), D0 (247.17 ± 3.63 g/fish) and D50 (247.89 ± 3.37 g/fish).

The whole-body composition of the experimental fish is presented in Table 5. Dietary replacement of FM by MFV meal did not affect (p>0.05) the moisture and body protein content of *C. gariepinus*. However, lipid deposition was significantly higher in fish fed with MFV-based-diets, whereas ash content significantly decreased (p<0.05).

Discussion

Water quality parameters were not significantly different between treatments and were within the acceptable ranges for *C. gariepinus* rearing [58]. The low zooplankton density observed in certain ponds could be attributed to fish predation [53]. Higher rotifers and copepods abundances reflect the optimal environmental conditions in ponds [51,53].

The present studies evaluate the potential of MFV to replace FM in *C. gariepinus* diet. To our known, there is no reliable study on the use of MFV as a protein source in diets for this fish. However, fisheries wastes recycling for fish farming is an economical and viable option for reducing environmental problems and simultaneously increasing animal protein production [35,59-61]. The results of this study indicated that it is possible to totally replace FM with MFV in African catfish diet without affecting growth performance, thus confirming previous studies findings that animal by-product meals are acceptable protein sources for replacement of fishmeal in catfish diet [2,6,11,19,29,34]. Previous studies have reported beneficial effects [34,62-64] but also adversely effects [65-69] of using MFV as protein sources in diets for several species. According to several studies, the poorest performance of fish fed alternative protein sources are due to the low feeding intake and low digestibility and imbalance of essential amino acids of diet [61,70]. The positive effect obtained in growth performance may be due to the increase protein digestibility and higher long chain polyunsaturated fatty acid content of MFV meal, as mentioned by Giri, et al. [11,32,34,71]. Indeed, according to Nwanna et al. [3,36,42], MFV meal is a good source of polyunsaturated fatty acid such as eicosapentaenoic acid and docosahexaenoic acid, which plays important roles in metabolism. Moreover, they are essential dietary nutrients as demonstrated in red sea bream Pagrus major and yellowtail Seriola quinqueradiata [72,73]. In this study, feed intake was significantly higher with fish fed D30 compared to that of fish fed other diets. This increasing in feed intake with fish fed diet D30 that contained some 15% FM could probably due to the presence of an adequate level of free amino acids in that diet containing lower level of MFV meal, as reported by Kotzamanis et al. [62]. Chotikachinda et al. [70] have reported significant inferior final weight, weight gain and specific growth rate in fish fed coppens diet compared to those of fish fed with the experimental diets, which is contrary with our findings. The weight gain and specific growth rate obtained here are higher than those reported by Sorensen et al. [30,33,74,75] with freshwater catfish Heteropneustes fossilis, Pacific threadfin Polydactylus sexfilis, *C. gariepinus* and Epinephelus fuscoguttatus fed respectively with fermented fish-offal, MFV meal, Agama agama meal and milkfish offal hydrolysate-based-diet. These results showed that MFV meal is better assimilated by fish species, including catfish than other those alternatives sources.

In the present study, whole body composition showed the inverse trend between lipid and ash content. Fish fed with D30 and D50 showed a significantly greater amount of body lipid and lower ash content, in comparison with those of fish fed coppens and D0. This trend was similar to that related in the earlier studies of Kristsanapuntu et al. [76] in red drum, Sciaenops ocellatus, and [32] in catfish *Clarias batrachus*. According to Luchtman et al. [32], the increased body lipid content may be due to increased energy content of diets containing MFV meal, which have a greater fat content (Table 1). The decreasing trend in ash content could be due to the reduction of FM and the inclusion of MFV meal in diet [77,78].

Conclusion

This study showed that up to 30% of marine fish viscera meal could be included in African catfish diet without adverse effects on growth performance and body protein composition. The use of marine fish viscera meal in *Clarias gariepinus* diet could reduce the cost of feed and increase the fish farmer incomes. This might enhance the expansion of the African catfish culture in Africa. We recommended that the further studies were carried out to determinate the optimal stocking density of *C. gariepinus* in order to improve the annual production.

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