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Chicken Viscera Meal as a Main Component in Diet for African Catfish *Clarias gariepinus* (Burchell 1822) Reared in Earthen Ponds

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Abstract The effects of replacement of fishmeal (FM) with chicken viscera meal (CVM) on growth, body composition and production of *Clarias gariepinus* fingerlings (initial body weight 11.3 ± 0.1 g) was studied for 90 days. Three isonitrogenous (43 % crude protein) and isoenergetic (20 KJ/g) diets were formulated to containing 0% (diet CMV0), 30% (diet CVM30) and 50% (diet CVM50) of chicken viscera meal (CVM), as FM replace. Diet CVM0, without CVM, acted as a control whereas the commercial diet coppens developed for *C. gariepinus* was used to validate the experimental design. Feed intake value (247.17-262.0 g) was similar in fish fed Coppens, CMV0 and CMV50, and significantly higher in fish fed CMV30 ($p<0.05$). Weight gain (range: 1900.0-2008.2 %), specific growth rate (3.33-3.40 % day^{-1}), protein efficiency ratio (1.95-2.09), yield (98.2-105.0 kg.are^{-1}) and annual production (398.4-425.7 kg.are^{-1}year^{-1}) were similar in fish fed Coppens, CMV0 and CMV30 diets ($p<0.05$). Values obtained for these parameters in fish fed CMV50 diets were significantly lower ($p>0.05$). Food conversion ratio (1.11-1.19) was significantly higher in fish fed CMV50 diets ($p<0.05$). Body protein content was similar in all the treatments ($p>0.05$). Lipid content in fish fed with CVM-based-diets was significantly higher, whereas ash significantly decreased ($p<0.05$). The study demonstrated that up to 30 % of CVM could be incorporated in the diets of *C. gariepinus* without negative effects on growth and whole body composition. Therefore, we recommended that CVM could be used to partially replace the expensive and imported FM.

Keywords: *Clarias gariepinus, fishmeal, chicken viscera meal, growth, body composition*

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1. Introduction

In the past four decades, fish farming has been rapidly expanded to meet the soaring global demand for seafood, which enhanced dramatically the production of aquaculture feeds. Global production of commercial aquaculture feeds increased from 7.6 million tons in 1995 to 29.2 million tons in 2008, with an average annual growth rate of 11%. It is expected to reach 51.0 million tons in 2015 and 71.0 million tons in 2020 [1]. Fishmeal is the main constituent of aquaculture feeds because of its high protein content, balanced amino acid profile, high essential fatty acids content, minerals and vitamins [2-8]. Nevertheless, the decrease in supply of stocks and increasing demand for aquaculture as well as degradation of natural fish populations has greatly increased the market price of fishmeal [8,9,10]. Moreover, high level of fishmeal in aquatic feeds may cause a series of environmental problems due to the high content of phosphorus. Therefore, the use of alternative animal protein sources in aquaculture feeds has become a research priority. In that way, some studies have evaluated some plant proteins as alternative sources of fishmeal in carnivorous diets because of their low price, consistent nutrient composition and supply [11]. However, findings showed poorer digestibility and adversely effects on growth performance due to their high carbohydrate content and imbalance amino acid composition [12]. Compared to plant-based proteins, chicken viscera is easily available at very low price throughout the year and have high protein value, low carbohydrate content, balanced amino acids profile, total digestible dry matter and lack of anti-nutritional factors [5,13-19]. Hence, chicken viscera are considered a probable substitute for fish meal in diets for a number of fish species [18-26].

African catfish *Clarias gariepinus* is a globally popular aquaculture species largely distributed throughout Africa and Asia [5,27,28,29,30,31]. 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 [27,28,31,32]. To achieve a good growth, catfish requires complete artificial feed of 35% to 45% protein level [28,33,34,35]. Recycling of poultry wastes into an acceptable source of animal protein in the catfish diet is a big challenge in the pursuit of sustained production. Nowadays, studies concerning the evaluation of poultry viscera meal in the *C. gariepinus* fingerlings diets are few. This study aimed to evaluate the potential of using chicken viscera meal as protein source in practical diets for African catfish reared in earthen pond.

2. Materials and Methods

2.1. Chicken Viscera Meal Production

Chicken viscera were collected fresh from the commercial processing industry “Agrisatch” located at Abomey-calavi suburb, and stored frozen (-20°C). The by-products were thawed, then heated on water vapor to remove fatty matter and dried in oven at 55°C for 48h. The dried-product was grounded and meal was stored in a refrigerator in plastic bag until use.

2.2. Fish and Experimental Design

The feeding experiment was carried out for 90 days in wetland area of Louho, at Porto-Novo, Benin. *C. gariepinus* fingerlings (average weight 11.3 ± 0.1 g) from Tonon fish farming foundation located at Calavi were randomly stocked into twelve earthen ponds (10m × 3m × 1m) at a density of 150 fish (5fish.m^{-2}) per pond. They were acclimated to experimental conditions for three days in ponds. Prior to start feeding, fish were fed a mixture of two experimental diets twice daily. At the start, the acclimated fish were deprived of feed for 24 h. Ponds were grouped into four triplicate and each was randomly assigned one experimental diet. All ponds were filled naturally from water table.

2.3. Experimental Diets and Feeding

Fish meal (FM) used in this study was *Sardinella aurita* meal. Slaughter house blood was collected from Calavi as fresh blood immediately after slaughtering ox. The blood was allowed to clot and only the clotted portion was collected and was immediately brought to the laboratory. At the laboratory, blood was heated in tap water and sun-dried for three days. Soybean oilcake (*Glycine max*), maize bran (*Zea mays*) and palm oil were purchased at the local market. Dried viscera, sun-dried blood, maize bran and soybean oilcake were grounded and separately stored in refrigerator (+4°C) until used. Analyses were done for proximate composition of all ingredients (Table 1) using standard method given in [36].

Three isoproteic (430 g/kg) and isoenergetic (20 kJg^{-1}) experimental diets (Table 2) were formulated to meet the protein and energy requirements of the juvenile catfish. Diet CVM0 without CVM and containing FM as the main animal protein acted as control diet. In diets CVM30 and CVM50, CVM was incorporated to replace partially and completely the FM. All the diets were compared to the commercial diet coppens in order to verify the validity of our facilities and experimental diets. Formulation and proximate composition of the experimental diets are shown in Table 2. The feed ingredients were ground in grinding mill to desired particle size, weighed and mixed thoroughly in a food mixer for 30 min. The hot water (about 30 % of diet dry weight) was progressively added to one kilogram of diet formulated and blended. The dough obtained was cut into paste-like and sun-dried at about 32-35°C for three days. After drying, the diets were broken into small particles (mm) and preserved in refrigerator (+4°C) until used. The proximate composition of all diets was analyzed in duplicate according to standard methods [36].

Table 1. Proximate composition (as % dry matter) of feeds ingredients

Ingredients	Dry matter	Crude protein	Crude lipid	Ash
Fish meal	92.0	66.0	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	30.0	13.2	3.7
Chicken viscera meal	90.9	35.0	22.0	6.3

Table 2. Formulation and proximate composition of experimental diets

Ingredients (%)	Diets			
	Coppens*	CVM0	CVM30	CVM50
Fish meal		30	15	0
Blood meal		23	23	23
Maize meal		30	20	10
Soybean oilcake		15	10	15
Chicken viscera meal		0	30	50
Palm oil		2	2	2
Proximate composition (%.MS)				
Dry matter	89.4	90.0	88.3	88.2
Crude protein	43.0	43.0	42.8	42.6
Lipid	13.0	10.8	12.0	14.0
Carbohydrate	34.1	31.0	32.4	33.7
Gross energy ($KJ.g^{-1}$)	21.2	20.2	20.0	20.8

*Characteristic: crude protein 43 %; crude fat 13 %; crude fiber 3.0 %; Ash 6.7 %; vitamin A, 15000 IU/kg; vitamin D_3, 2000 IU/kg; vitamin E, 200 mg/kg; vitamin C, (stable) 150 mg/kg; phosphorus 0.9 %; calcium 0.9%; sodium 0.2%; preservatives E280; antioxidants E324, E321. Composition: soya dehulled, extracted, toasted, wheat, rape seed, extracted, pea protein, maize gluten, premix. Produced by Coppens International bv, PO Box 534, 5700 AM Helmond, Holland.

During the feeding trial, fish were fed with hand to apparent satiation at 09:00 and 17:00 hours daily, taking care to stop feeding when fish stopped eating. In each fortnight, thirty percent (40 %) of the stocked fish in each pond were sampled out with a seine net (12.7 mm mesh size) and weighed for growth control [37,38].

2.4. Water Quality Parameter

Temperature, dissolved oxygen, hydrogen ion concentration (pH), and total dissolved solid (TDS) were monitored at a deep of 10 cm using the multiparameter HANNA HI-9828 instrument. The transparency was measured using Secchi disk. Nutrients such as nitrite and ammonium were determined by cadmium reduction and phenate methods respectively. The abundance of zooplankton was estimated by counting samples in a Dolfus cell under a binocular magnifying glass (x 40).

2.5. Biochemical Analysis

Hundred chosen fish were randomly sampled from the initial population to determine initial carcass composition. For final carcass composition analysis, twenty fish were selected at random from each pond. The fish samples and diets were analyzed according to standard method [36] for dry matter and total ash. Crude protein was determined by the Kjeldhal procedure with the Kjeltec System (Tecator, Sweden). Lipid determination was made by chloroform–methanol method [39].

2.6. Calculations

At the end of the experiment, growth performance and feed utilization were assessed by determination of Weight gain (WG), Specific growth ratio (SGR), Apparent feed conversion ratio (AFCR), protein efficiency ratio (PER), survival (S), yield (Y) and production (P) as below:

$$S\ (\%) = 100 \times (\text{final count})/(\text{initial count})$$

$$WG\ (g) = 100 * (FB - IB)/IB$$

$$SGR\ (\%/day) = 100\left[\ln\left(w_f\right) - \ln\left(w_i\right)\right]/\Delta t$$

$$DWG\ (g/j) = \left(w_f - w_i\right)/T$$

$$AFCR = TFI\ /\ (FB - IB)$$

$$PER = (FB - IB)/DPI$$

$$Y(kg/are) = (FB - IB)/S$$

$$P(kg/are/year) = ([FB - IB)/S] \times 365)/\Delta t$$

where w_i and w_f = initial and final mean body mass (g); Δt is the duration of experiment (days); FB 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.

2.7. 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 test and were considered to be significant when P-values were < 0.05. Analysis of covariance (ANCOVA) was used to compare growth performance in fish fed experimental diets using days as covariate. Before doing the analyses, homogeneity of variance was verified using [40]. and abnormal data were log-transformed. All analyses were done using the statistical package SPSS version 22.0 for windows (SPSS, Chicago, Illinois, USA).

3. Results

Values for water quality parameters and zooplankton density (number l^{-1}) in different types of ponds were shown in Table 3. Values for water transparency, temperature, pH, dissolved oxygen, total dissolved solid, nitrite and ammonium were similar in all treatments (p>0.05). Significant differences in absolute density and zooplankton abundance were obtained. Higher absolute density value was obtained in ponds receiving coppens diets (1531 ± 26 ind.l^{-1}), while lower values were obtained in ponds receiving CVM0 diets (600 ± 27ind.l^{-1}). Copepods and rotifers were the most abundant zooplankton groups in all ponds.

Table 3. Water quality parameters and zooplankton density (number/l) in different treatments during 90 days trial

Parameters	Diets			
	Coppens	CVM0	CVM30	CVM50
1. Water quality				
Transparency (cm)	15.6 ± 2.8	16.4 ± 2.4	17.00 ± 1.02	15.5 ± 0.92
Temperature (°C)	29.73 ± 1.76	29.29 ± 1.47	29.95 ± 1.72	29.53 ± 1.46
pH	6.01 ± 0.50	5.76 ± 0.23	5.87 ± 0.21	6.14 ± 0.53
Dissolved oxygen (mg.L^{-1})	4.26 ± 2.48	4.17 ± 1.67	3.53 ± 1.86	4.95 ± 3.28
TDS (ppm)	50.28 ± 6.54	52.00 ± 8.62	54.33 ± 8.46	50.78 ± 7.64
Nitrite (mg l^{-1})	0.05 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.04 ± 0.02
Ammonium (mg l^{-1})	0.005 ± 0.002	0.006 ± 0.002	0.005 ± 0.002	0.005 ± 0.001
2. Zooplankton (number l^{-1})	1531 ± 26[a]	600 ± 27[d]	778 ± 268[b]	747 ± 125[c]
(%) Copepods	48.98	56.25	44.00	58.75
(%) Rotifers	35.67	38.96	52.52	38.35
(%) Cladocerans	15.35	4.79	3.48	2.90

Means values within the same line following by different superscript are significantly different (p<0.05). Coppens, Catfish commercial diet; CVM0, diet with 0% chicken viscera meal; CVM30, diet with 30% chicken viscera meal; CVM50, diet with 50% chicken viscera meal.

Changes in fortnight mean weight of experimental fish showed growth overtime (Figure 1). Growth and feed performance of *C. gariepinus* fed chicken viscera-based-diets for 90 days trial are presented in Table 4. Survival rate were greater than 90 %, indicating that fish were reared in good conditions. Fish fed CVM30 diet had a higher feed consumption (262.0 ± 3.4 g) than those fed with coppens (248.3 ± 2.7 g), CVM0 (247.2 ± 2.6 g) and CVM50 (251.7 ± 3.2 g).Values for final weight (range: 225.6 – 237.8 g), weight gain (range: 1900.0-2008.2 %), specific growth rate (range: 3.33 – 3.40 % day^{-1}), AFCR (range: 1.11 – 1.19), PER (range: 1.95 – 2.09), yield (range: 98.2 – 105.0 kg are^{-1}) and annual production (range: 398.4 – 425.7 kg.are^{-1}year^{-1}) were similar in fish fed coppens, CVM0 and CVM30 (p>0.05). Fish fed CVM50 show significantly lower performances (p>0.05). The ANCOVA analysis of variation in weight using days as covariates was evaluated at 45 days. As shown in Figure 2, the results followed the trend obtained with the *post hoc* test of ANOVA, thus confirming that Coppens, CVM0 and CVM30 have similar growth performance, which is higher to that of CVM50.

Whole-body composition of *C. gariepinus* fed the experimental are showed in Table 5. The moisture (range: 10.35 - 10.77 %) and crude protein (range: 60.59 - 61.42 %) of fish fed the test diets were not significantly different (p>0.05). Lipid deposition (range: 13.19 - 18.53%) in fish fed chicken viscera-based-diets is significantly higher, whereas Ash content (10.42 ± 0.89 - 14.53 ± 0.50) decreased with increasing CVM in diets (p<0.05).

Table 4. Growth and feed performances of *C. gariepinus* fed graded levels of chicken viscera meal for 90 days

Parameters	Coppens	CVM0	CVM30	CVM50
Initial weight (g)	11.3 ± 0.1	11.3 ± 0.1	11.3 ± 0.1	11.3 ± 0.1
Survival (%)	90.00 ± 2.89	91.00 ± 2.47	90.67 ± 1.47	91.68 ± 1.15
Final weight (g)	234.1 ± 6.7^{a}	233.3 ± 6.5^{a}	237.8 ± 5.2^{a}	225.6 ± 3.5^{b}
Condition factor	0.96 ± 0.11^{ab}	0.95 ± 0.09^{b}	0.97 ± 0.08^{a}	0.93 ± 0.04^{c}
Feed intake (g fish^{-1})	248.30 ± 2.67^{b}	247.17 ± 2.63^{b}	262.0 ± 3.41^{a}	251.67 ± 3.23^{b}
Specific growth rate (% days^{-1})	3.37 ± 0.03^{ab}	3.36 ± 0.02^{ab}	3.40 ± 0.03^{a}	3.33 ± 0.02^{b}
Weight gain (%)	1971.7 ± 60^{ab}	1964.6 ± 57.5^{ab}	2008.2 ± 46.1^{a}	1900.0 ± 31.0^{b}
Apparent feed conversion ratio	1.12 ± 0.03^{b}	1.11 ± 0.03^{b}	1.13 ± 0.02^{b}	1.19 ± 0.02^{a}
Protein efficiency ratio	2.08 ± 0.04^{a}	2.09 ± 0.03^{a}	2.05 ± 0.03^{a}	1.95 ± 0.02^{b}
Yield (kg are^{-1})	100.3 ± 3.0^{ab}	101.0 ± 3.0^{ab}	105.0 ± 2.4^{a}	98.2 ± 1.6^{b}
Production (kg are^{-1}year^{-1})	406.6 ± 12.3^{ab}	409.7 ±12.0^{ab}	425.7 ± 9.8^{a}	398.4 ± 6.5^{b}

Coppens, Catfish commercial diet; CVM0, 0% chicken viscera meal; CVM30, 30% chicken viscera meal; CVM50, 50% chicken viscera meal. Means values in the same row having different superscript are significantly different (p<0.05).

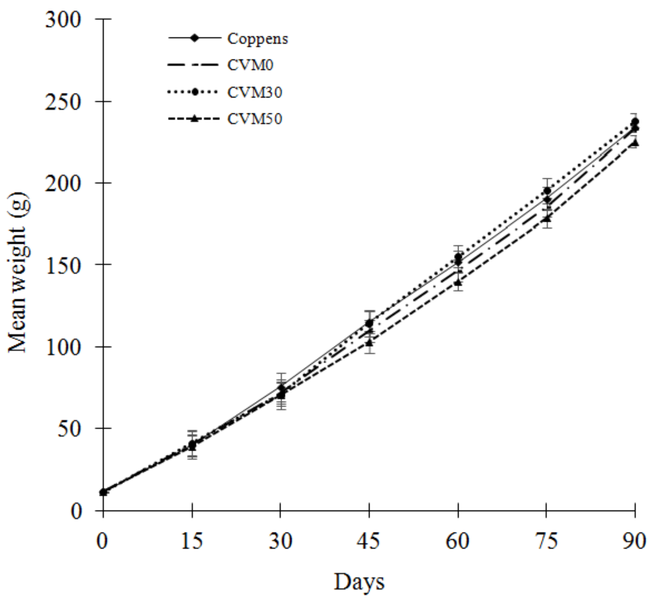

Figure 1. Fortnightly mean weight changes of *Clarias gariepinus* fed chicken-based-diets

Table 5. Whole-body proximate composition (% of dry matter) of *Clarias gariepinus* fed chicken viscera-based-diets for 90 days

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^{b}	14.53 ± 0.50^{a}
CVM0	10.55 ± 0.28	61.42 ± 0.98	14.32 ± 0.78^{b}	14.53 ± 0.10^{a}
CVM30	10.45 ± 0.03	61.41 ± 0.28	17.37 ± 0.59^{ab}	10.81 ± 0.16^{b}
CVM50	10.35 ± 0.20	60.59 ± 0.08	18.53 ± 0.76^{a}	10.42 ± 0.89^{b}

Coppens, Catfish commercial diet; CVM0, 0% chicken viscera meal; CVM30, 30% chicken viscera meal; CVM50, 50% chicken viscera meal. Means values having different superscript are significantly different (p<0.05).

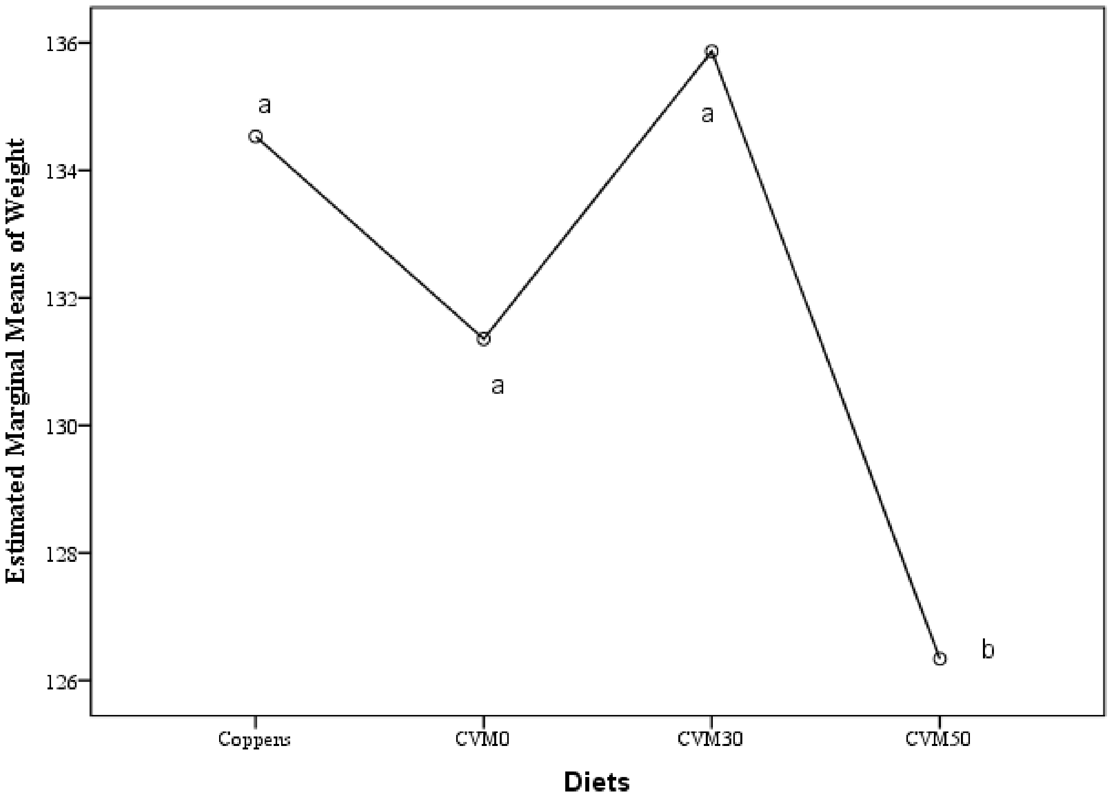

Figure 2. Variation in weight with different experimental diets (Covariates appearing are evaluated at 45 days)

4. Discussion

Water quality parameters in all types of ponds were within the acceptable range for African catfish *Clarias gariepinus* rearing, as reported by [41]. The low zooplankton density observed in certain ponds could be attributed to fish predation [37] or probably to a better utilization of certain feed fed namely CVM0, CVM30 and CVM50, leading to lower wastes discharge that failed to boost the natural food in ponds receiving these diets. Higher rotifers and copepods abundances reflect the optimal environmental conditions in ponds [42].

In this study, although the different groups of experimental fish had similar initial mean weight and were fed with isonitrogenous and isoenergetic diets, *Clarias gariepinus* showed significant different in final weight gain, specific growth rate, feed intake, feed conversion ratio and protein efficiency ratio. Higher substitution levels of chicken viscera meal resulted in reduced growth performance, feed utilization efficiency and production. Similar results were reported for *Carassius auratus gibelio* [43,44], *Clarias gariepinus* [5], *Clarias batrachus* [25,26,45], grass carp *Ctenopharyngodon idella* fry [46], *Cirrhinus mirigala* [41], *Pelodiscus sinensis* [47] and *Lutjanus guttatus* [4] fed on diets containing graded poultry viscera meal. According to [25,45,47,48], this poor growth performance at high inclusion level may be due to the presence of undigested grain and their by-products from the poultry diet, and the imbalanced amino acid content of the diet without fishmeal. But although the feed intake was significantly higher in fish fed 30% chicken viscera, growth performances and feed utilization parameter were similar until 30 % CVM in diets, thus confirming that FM has superior nutritional values than other animal proteins [49] and plant proteins [50], because of its well-balanced amino acid composition and their bio-availability [7,8,51] that influence performance in animals [52]. Similar results were found by [47] and [4,53] in *Pelodiscus sinensis, Orechromis niloticus* and *Lutjanus guttatus*, respectively. The survival rate during the experiment was greater than 92%, indicating that fish has grown in good experimental conditions.

The replacement of FM by CVM show lower AFCR and the PER was improved. The highest AFCR and lower PER were observed in fish fed CMV50 diets. According to [5]., this is due to the ash and carbohydrate content in CVM, by producing a faster gut transit rate, resulting in increased feed intake, associated with poor growth performance and feed efficiency. Same results have been reported by [54] who found that higher feed intake and lower feed efficiency in juvenile rainbow trout can be attributable to higher quantity of ash in diets. Also, feed intake and growth performance decreasing trends were found in *Clarias gariepinus* [5,55], *Clarias batracus* [25,44] and *Anabas testudineus* [19] at high inclusion levels of poultry viscera meal in diets.

Yield of fish fed experimental diets was not significant different until 30% CVM in diets, suggesting that coppens diet and diets containing 0 and 30 % of chicken viscera meal were well-balanced in indispensable amino acid and well assimilated by *C. gariepinus*.

The results of whole-body composition of fish have shown similar protein content in all treatments. However, lipid content increased, probably caused by the CVM level in diets. These results were in agreement with [5,25,56,57], who reported that replacement of FM by poultry viscera meal in diets did not affect the body protein content, but increase whole-body lipid content of fish. The ash content decreased with the CVM level in the diets, which corroborate the observation of [25], and [57] in *Clarias batrachus* juveniles.

5. Conclusion

The present study clearly demonstrated that chicken viscera have a good nutritive value for African catfish growth. It could be incorporated up to 30 % level in *C. gariepinus* diets without adverse effects on growth, feed utilization and body protein content. Chicken viscera are available in Benin and can be obtained throughout the year. Thereby, the use of chicken viscera can substantially reduce feed cost and increased profits. This might permit the development of that fish culture in Benin. However, we recommend further studies in order to determinate the optimal stocking density of *C. gariepinus* so as to improve production and generate maximal profit.

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