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Vincent Oké
Laboratory of Ecology and Aquatic Ecosystem Management, Department of Zoology, Faculty of Sciences and Technics, University of Abomey-Calavi, Abomey-Calavi, Republic of Benin

Youssouf Abou
Laboratory of Ecology and Aquatic Ecosystem Management, Department of Zoology, Faculty of Sciences and Technics, University of Abomey-Calavi, Abomey-Calavi, Republic of Benin

Correspondence
Youssouf Abou
Laboratory of Ecology and Aquatic Ecosystem Management, Department of Zoology, Faculty of Sciences and Technics, University of Abomey-Calavi, Abomey-Calavi, Republic of Benin

Evaluation of marine fish viscera as a protein source in diet for juvenile African catfish *Clarias gariepinus* (Burchell, 1822) reared in concrete tanks

Vincent Oké and Youssouf Abou

Abstract
The goal of this study was to evaluate the suitability of marine fish viscera (MFV) as an alternative protein in diet for juvenile *Clarias gariepinus*. Three isonitrogenous (43% crude protein) and isoenergetic (gross energy 20 $kJ.kg^{-1}$) diets were formulated to contain 0% (D0, control diet), 30% (D30) and 50% (D50) of MFV, as fishmeal (FM) substitutes. The diets D0, D30 and D50 were compared with the reference commercial diet Coppens® developed for African catfish. Fish (initial body weight: 8.0 ± 0.3g) stocked at fifty-five per tank where fed at satiation in twelve (4 x 3 replicates) tanks for 42-day in a recirculating water system. At the end of experiment, final weight (96.6 g - 111.0 g), daily weight gain (2.1 g/day - 2.5 g/day), Specific Growth Rate (5.7%/day - 6.3%/day), feed conversion ratio (1.1-1.3), protein efficiency ratio (1.8-2.2), yield (4.7 kg/m^3 - 6.1 kg/m^3) and annual production (41.1 Kg/m^3/year - 53.1 Kg/m^3/year) did not vary significantly until 30% MFV in diet ($P > 0$). Values in fish fed 50% MFV were significantly lower ($P < 0$). Cost-benefit analysis show a considerable reduction in cost of production of one kilogram fish, to about 79.34% and 76.03% with fish fed 30% and 50% MFV, respectively, compared to commercial diet. Thus, the MFV should be included up to 30% in diet for *C. gariepinus* juvenile without negative influence on growth performance, feed utilization and production. The results encourage more investigations in promoting of that environmental-friendly ingredient in the production of *C. gariepinus* in Benin small-scale farms.

Keywords: Catfish *Clarias gariepinus*, fish meal, marine fish viscera, diet, replacement

Introduction
African catfish *Clarias gariepinus* is a major warm water aquaculture species in Africa and Asia (Khan and Abidi, 2011) [29]. It is an excellent species for intensive culture because of their tolerance to poor water quality, ability to maintain high growth at high densities, resistance to diseases and ability to accept readily artificial feed (Nyina-wamwiza *et al.,* 2007) [38]. C. *gariepinus* is also appreciated compared to other aquaculture species by the fish farmers because of its growth performance, good meat quality and relative high market price. The yields of African catfish from pond could be as much as 2.5 times higher than those of Tilapia (Hogendoorn 1983) [26]. However, African catfish production is quite limited in most African countries because the adequate feed for juvenile on-growing is scarce and more expensive (Nyina-wamwiza *et al.,* 2007) [38]. Commercial production of catfish relies on commercial diets formulation with high level of fishmeal. Indeed, fishmeal is ideal protein source in aquafeed due to high protein content, well-balanced indispensable amino acid profile and better protein digestibility (Mambrini & Kaushik, 1995; Giri *et al*., 2010) [33, 14]. The stagnation or declining trend of fisheries capture has led to unavailable and expensive of fishmeal. Limited availability of fishmeal will subsequently represent a constraint for the development of aquaculture production. Therefore, it is necessary to substitute the fish meal used by inexpensive alternative protein sources. In that way, appropriate use of local protein by-products could reduce feed costs by saving transportation expensive, and has the potential to enhance environmental and economic sustainability (Ju *et al.*, 2013) [28].
Fish processing generate significant quantities of by-products in market places. Generally, fish by-products are in majority constituted of viscera, which likely have similar nutritional qualities as the fishmeal currently used in aqua-feeds (Bureau *et al*., 2000; Sun *et al.,* 2002; Chotikachinda *et al.,* 2013; Ju *et al.,* 2013) [5, 45, 9, 28].

Fish viscera include highly digestible, well-balanced proteins, significant quantities of lipids with long chain, highly unsaturated n-3 fatty acids, as well as taurine and others growth factors (Ju *et al.,* 2013) [28]. According to the same authors, fish viscera have low anti-nutritional factors (e.g. phytase) and carbohydrate. Recycling of these wastes into an acceptable source of animal protein in diet for fish is a big challenge in the sustainable production of inexpensive catfish *Clarias gariepinus* diets. Previous studies revealed satisfactory growth and feed utilization responses through replacement of fishmeal by fish viscera in diets for several species. *Thynnus thynnus* viscera meal, *Chanos chanos* viscera hydrolysate, dried fish viscera, *Loligo vulgaris* viscera meal, *Raja rhina* and *Anoplopoma fimbria* viscera meals have been evaluated in red drum *Sciaenops ocellatus* (Li *et al.,* 2004; Whiteman & Gatlin 2005) [30, 53], *Epinephelus fuscoguttatus* (Mammauag & Ragaza 2016) [32], *Clarias batrachus* (Giri *et al.,* 2000a, 2010; Gupta *et al.,* 2013) [15, 14, 21], Asian sea bass *Lates calcarifer* (Chotikachinda *et al.,* 2013) [9], Japanese seabass *Lateolabrax japonica* (Mai *et al.,* 2006) [31], Pacific threadfin *Polydactylus sexfils* (Forster & Babbitt 2005) [13], Japanese flounder *Paralichthys olivaceus* (Uyan *et al.,* 2006) [50], tilapia *Oreochromis mossambicus* and carp *Cyprinus carpio* (Gümüs *et al.,* 2009, 2011; Tekinay *et al.,* 2009; Saïdi *et al.,* 2010) [20, 19, 47, 42], shrimp *Litopenaeus vannamei* (Hernandez *et al.,* 2004) [25], and spotted rose snapper *Lutjanus guttatus* (Hernandez *et al.,* 2014) [24] with good results.

As far as we know, information on replacement of fishmeal by marine fish viscera in diet for C. *gariepinus* is not available. This study was carried out to evaluate the effects of partial and complete substitution of dietary fishmeal with fish marine viscera on growth performance and feed utilization of African catfish *C. gariepinus*.

Materials and Methods

Feed ingredients, diet formulation and pellet preparation

Marine fish viscera was collected fresh from the fishing port of Cotonou just after dressing and stored frozen (-20°C) until used. Fish by-product was autoclaved and ground to make the product pathogen free, and stored in refrigerator (+4°C). Fish meal was made from dried *Sardinella aurita* purchased from the market place. The blood was collected from Calavi slaughterhouse. The other ingredients such as maize (*Zea maize*), soybean (*Glycine max*) and palm oil were purchased at the local market. All ingredients were analysed for proximate composition using standard method given in AOAC (2005) [4]. The proximate composition is giving in Table 1.

Three isoproteic (430 g kg^{-1} crude protein) and isoenergetic (20 kJ kg^{-1} gross energy) diets were formulated to satisfy the dietary protein requirements of catfish *C. gariepinus* (Hecht *et al.,* 1997; Nahar *et al.,* 2000) [22, 35]. Fish meal level of the control diet D0 was 300 g kg^{-1}. In the remaining diets D30 and D50, fish meal was replaced partially and completely with fish viscera. The commercial diet (coppens) and D0 were used as control diets in order to validate our experimental facilities and diets. Formulation, proximate composition and gross energy content of the test diets are presented in Table 2.

The feed ingredients were ground, weighed and mixed thoroughly with fish viscera. Tap water was progressively added in diet contain high level of fish meal (control diet) until obtained a dough. The dough was cut into paste and sun-dried for about three days at 32-35 °C. After drying, the diet was broken into small particles (1-2 mm) and preserved in refrigerator (+ 4 °C) until used.

Table 1: Proximate composition (expressed as per cent dry matter) of fed ingredients

Ingredients	Dry matter	Crude protein	Crude lipid	Ash
Fish meal	92.0	66.0	7.88	15.77
Blood meal	90.9	71.9	1.70	6.40
Maize bran	91.4	6.2	3.10	1.40
Soybean oilcake	94.8	30	13.2	3.70
Fish viscera	27.0	38.8	39.0	7.0

Table 2: Formulation and proximate composition of the experimental diets used for *Clarias gariepinus* in concrete tanks for 42 days. D0, is the control diet with 0% MFV, D30 diet with 30% MFV, D50 diet with 50% MFV.

Diets	Coppens*	D0	D30	D50
Ingredients (g.kg^{-1} of Dry matter)				
Fish meal		30	15	0
Blood meal		23	23	23
Maize		30	20	10
Soybean meal		15	10	15
Fish viscera		0	30	50
Palm oil		2	2	2
Composition (% Dry matter)				
Dry matter	89.4	90.0	88.4	90.3
Crude protein	43.0	43.0	43.3	43.2
Crude lipid	13.0	10.8	12.3	12.9
Carbohydrate	34.1	31.0	31.8	31.2
Crude energy** (KJ.g^{-1})	21.2	20.2	20.6	20.7

*Characteristic of the coppens : crude protein 43%; crude fat 13%; crude fibre 3.0%; Ash 6.7%; vit A 15000 IU/kg; Vit D3 2000 IU/kg; vit E 200 mg/kg; vit C (stable) 150 mg/kg; phosphorus o.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

**Gross energy was calculated on the basis of 23.7 kJg$^{-1}{}_{protein;}$ 39.5 kJg$^{-1}{}_{lipid;}$ and 17.2 kJg$^{-1}{}_{carbohydrate}$ according to Guillaume *et al.* (1999) [18].

Fish, husbandry and feeding

The experimental fish were obtained from the Tonon Cossi Gilbert fish farming foundation (N06°25'39.1", E002°16'27.4") located at Ouèdo village and were transported to the aquaculture research station at University of Abomey-Calavi, Benin (West Africa). Thousand (1000) juveniles of C. *gariepinus* (initial mean weigh 8.0 ± 0.3 g) were acclimated to the experimental conditions in concrete tanks for 2 weeks, during which time all fish was fed a mixture of experimental diets in equal proportions, to habituate them to the locally formulated feed.

The experiment was carried out in a recirculating system including twelve 0.9 m^3 experimental concretes tanks. The useful water level was about 0.6 m^3 in each tank. Water recirculating through mechanical and biological filters was pumped into each tank at a flow rate of 2 L min^{-1}. Fish were randomly distributed at 55 fish per tank. The experimental tanks were randomly assigned to four groups; each in three replicates (4 × 3 tanks) was attributed to one of the diets. Fish were hand-fed to satiation at 09:00 and 17:00 hours daily. Care was taken to stop feeding as soon as they stopped eating. Water parameters were monitored biweekly, as follows: temperature and dissolved oxygen were measured with an oxythermometer WTW Oxi 197/Set, and pH was recorded using a pH meter WTW pH 330/Set-0.

Calculations

The feed intake (FI), weight gain (WG), specific growth ratio (SGR), daily weight gain (DWG), feed conversion ratio (FCR), protein efficiency ratio (PER), yield (Y), production (P) and survival (S) were evaluated as below:

$(WG, \%) = 100 \times (w_f - w_i) / w_i$

$(SGR, \%\ day^{-1}) = 100 \times [\ln(w_f) - \ln(w_i)]\ \Delta t^{-1}$

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

$(DWG, g/j) = (w_f - w_i)\ T^{-1}$

$(FCR) = TFI\ (FB - IB)^{-1}$

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

$Y (kg\ m^{-3}) = (FB - IB)/V$

$P (kg\ m^{-3}\ year^{-1}) = ([FB - IB)\ V^{-1}] \times 365)\ \Delta t^{-1}$

Where w_i and w_f are initial and final mean body mass (g); FB and IB are the final and initial biomass per tank (g); Δt is the duration of experiment (days); TFI the total food intake (g); DPI the dietary protein intake; V is the volume of water.

Statistical analysis

All data were subjected to a One-Way Analysis of Variance (ANOVA) and Least Significant Difference (LSD) test to determine if significant differences occurred among the treatments. Variance homogeneity was first checked by the Hartley test (Dagnelie, 1975) [10]. Effects with a probability of P-values < 0.05 were considered significant. Before analyses, all data except initial body weight were normalized by log transformation. All analyses were done using the statistical package SPSS version 22.0 for windows (SPSS, Chicago, Illinois, USA).

Results

Water quality monitoring

All the water quality parameters were within the acceptable range for African catfish *Clarias gariepinus*. The water temperature ranged from 26.8 to 28.0 °C, dissolved oxygen from 5.6 to 6.23 mg.L^{-1} and pH from 5.9 to 6.3.

Growth performance and feed efficiency

As showed in Table 3, survival rate ranged from 92.5 to 97.5% in all treatments (p>0.05). There were no significant different in the total feed intake among the dietary treatment (p>0.05). Final body weight (range: 96.3-111.01 g), daily weight gain (range: 2.1-2.5 g) and specific growth rate (range: 5.7-6.3%.day^{-1}) of fish fed coppens, D0 and D30 were similar (p>0.05). Values obtained in fish fed D50 are significantly lower (p<0.05). Same trends of variation were found in feed conversion ratio (range: 1.1-1.3) and protein efficiency rate (range: 1.8-2.2). The yield and annual production values ranged from 4.7 to 6.1 Kg.m^{-3} and 41.1 to 53.1 Kg.m^{-3}year^{-1} respectively, with significant difference in fish fed D50 (p<0.05).

Economic analysis

The ingredients and experimental feed cost for producing one kg weight gain of fish were summarized in Table 4. Economic evaluation take into account the feed costs, the costs of one kg gain in weight and its ratio to that of coppens group. The results indicated that incorporation of marine fish viscera in catfish diets as an unconventional ingredient to replace fishmeal decreased feed costs by 79.0 and 80.0% for the D30 and D50 compared to coppens. Costs of one kg gain in weight were 0.58 US$ for fish fed 30% viscera and 0.50 US$ for fish fed 50% viscera.

Table 3: Growth parameters, feed efficiency and production of *Clarias gariepinus* fed marine viscera based diet.

Parameters	Coppens	D0	D30	D50
Initial weight (g)	8.0 ± 0.3	8.0 ± 0.3	8.0 ± 0.3	8.0 ± 0.3
Survival	94.4 ± 5.6	97.5 ± 2.2	95.0 ± 1.1	92.5 ± 6.5
Final weight (g)	111.01 ± 5.7^a	109.6 ± 3.3^a	109.3 ± 10.7^a	96.3 ± 6.2^b
Daily weight gain (g)	2.5 ± 0.1^a	2.4 ± 0.1^a	2.4 ± 0.2^a	2.1 ± 0.1^b
Weight gain (%)	1287.6 ± 112.6^a	1270 ± 22.6^a	1266.3 ± 95.8^a	1103.8 ± 81.4^b
Specific growth rate (%.day^{-1})	6.3 ± 0.1^a	6.2 ± 0.1^a	6.2 ± 0.2^a	5.7 ± 0.1^b
Feed intake (g)	5195 ± 360	5800 ± 200	5900 ± 265	5333 ± 379
Feed conversion ratio	1.1 ± 0.1^a	1.1 ± 0.0^a	1.1 ± 0.0^a	1.3 ± 0.0^b
Protein efficiency ratio	2.2 ± 0.1^a	2.1 ± 0.0^a	2.1 ± 0.0^a	1.8 ± 0.1^b
Yield (kg m^{-3})	5.4 ± 0.7^a	5.8 ± 0.1^a	6.1 ± 0.5^a	4.7 ± 0.5^b
Production (kg m^{-3} year^{-1})	47.1 ± 5.9^a	50.6 ± 1.1^a	53.1 ± 4.6^a	41.1 ± 3.9^b

^{a,b,c,} In each line, means without or with the same letters as superscripts are not significantly different (p> 0.05)

Table 4: Summary of ingredients and experimental feed costs of producing one kg weight gain of *Clarias gariepinus.*

Ingredients (%)	Experimental diets				Price (US$ kg^{-1})
	Coppens	D0	D30	D50	
Fish meal		30	15	0	0.51
Fish viscera		0	30	50	0.25*
Blood meal		23	23	23	0.18**
Maize bran meal		30	20	10	0.26
Soybean meal		15	10	15	0.67
Oil palm		2	2	2	1.36***
Total		100	100	100	
Feed cost without charge cost (US$ kg^{-1})		0.40	0.34	0.32	
Charge cost (US$ kg^{-1})		0.12	0.12	0.12	
Feed cost (US$ kg^{-1})	2.20	0.52	0.46	0.44	

Relative to coppens (%)	100	23.6	21.0	20.0	
Decrease in feed costs (%)	0	76.4	79.0	80.0	
Cost of feed used (US$ kg^{-1} weight gain)¶	2.42	0.58	0.50	0.58	
Relative to coppens (%)	100	23.97	20.66	23.97	
Decreased feed costs (US$ kg^{-1} weight gain)	0	76.03	79.34	76.03	

*****Concerning the collect, transport and processing cost**, ****Equivalent of transport and processing cost**, *****Cost per liter. 1 US$ = 586.69 FCFA at present, ¶ cost of feed used (US$/kg weight gain) = FCR × cost of kg feed.

Discussion

In this study, the water temperature values were in the range of optimal temperature for the growth of *Clarias gariepinus* (Degani *et al.,* 1989; Goddard, 1996) [11, 17]. Also, the dissolved oxygen concentrations measured was in the optimum recommended by Viveen *et al.* (1985) [52] for culturing *C. gariepinus.* The mean value of pH was slightly acidic but tolerable for the species (Henken *et al.,* 1986; Huisman and Richter, 1987) [23, 27].

The experimental diets were well accepted by the catfish, as no rejection of the feed was observed throughout the feeding trial. In consequence, growth performance and feed conversion ratio were only affected by diets. Fish were fed to satiation and resulted in an increased feed intake in all diets. The increased feed intake in fish fed D30 and D50 could be due to the presence of many free amino acids, which improved flavour and taste of those diets, as observed by Giri *et al.* (2000a) [15], Gupta *et al.* (2013) [21] and Tabinda *et al.* (2013) [46] in *Clarias batrachus* L. and grass carp *Cirrhinus mirigala* fry, respectively. These findings revealed that dried fish viscera improve the free amino acids content in diet.

Similar growth performance values were obtained in fish fed with coppens, D0 and D30. This confirms the previously studies of Giri *et al.* (2000a; 2010) [15, 14] and Gupta *et al.* (2013) [21], who found that fish meal could be partially replaced by dried fish and chicken viscera in diets for *Clarias batrachus* fingerlings, without negatively affected the growth and feed conversion ratio. Same trends of growth performance were obtained with *Onchorhynchus mykiss* (Sugiura *et al.,* 2000) [43], *Heteropneustes fossilis* (Mondal *et al.,* 2008) [34] and *Polydactylus sexfilis* (Ju *et al.,* 2013) [28]. The results were also comparable to those reported in several studies with the same purpose of replacing of fish meal by animal protein source in *C. gariepinus* diets (Goda *et al.,* 2007; Soltan *et al.,* 2008; El-Husseiny *et al.,* 2013; Adewolu *et al.,* 2010; Abdelhadi *et al.,* 2010; Tiamiyu *et al.,* 2013; Chor *et al.,* 2013) [16, 44, 12, 3, 1, 48, 8], thus confirming that fish viscera-diet formulated is valuable for this fish in the rearing conditions. The lowest growth performance recorded in fish fed diet D50 compared to D30 can be due to the high amount of MFV. This observation is supported by the results of Cahu *et al.* (1999) [7] and Ovissipour *et al.* (2012) [41] who reported that the presence of high levels of free amino acids depress the maturation process of pancreatic cells in fish, as well as the digestive enzymes secretion.

The results obtained here in term of weight gain and specific growth rate were higher than those of Nwanna (2003) [36], Adewolu *et al.* (2010) [3], Chor *et al.* (2013) [8], who reported 334 to 382% and 1.75 to 1.87%/day; 149 to 202.73% and 2.67 to 3.40%/day; 14.04 to 999.62% and 0.47 to 2.08%/day with *C. gariepinus* fed fermented shrimp head waste meal, animal protein mixture and feather meal, respectively.

The feed conversion ratio values obtained in this study were similar to those reported by Ovie and Adejeyan (2010) [40] in *C. gariepinus* fed the garden snail (*Limicolaria* Spp.), thus suggesting that our experimental diets are well assimilated by *C. gariepinus* in the rearing conditions. In contrary, our findings are lower than 2.61-2.96 and 1.24-3.08 reported by Tiamiyu *et al.* (2013) [48] and Chor *et al.* (2013) [8] for *Clarias gariepinus* fed with varying levels of *Agama agama* meal and feather meal, respectively.

The protein efficiency ratio values from this study were similar to those reported by Giri *et al.* (2000a) [15] and Gupta *et al.* (2013) [21] when feeding Asian catfish, *Clarias batrachus* fed with dried fish and chicken viscera at 30% level in diet. They are higher than the values obtained with inclusion of 0 to 50% (Hernandez *et al.,* 2014) [24] and 0 to 40% (Valle *et al.,* 2015) [51] of fish hydrolysate in diet for juvenile spotted rose snapper, *Lutjanus guttatus* and *Litopenaeus vannamei* post larvae, respectively.

The results of fish survival rate are high and corroborate with the results of Toko *et al.* (2007) [49] and Ovissipour (2012) [41], who have not observed any significant differences in *Clarias gariepinus* and Persian sturgeon *Acipenser persicus* L. fed with graded levels of soybean and cottonseed meals, and tuna viscera protein hydrolysate, respectively. Chor *et al.* (2013) [8] have reported a low survival rate in African catfish *C. gariepinus* fry fed feather meal diet compare with fish viscera diet.

This lower mortality and high growth performances leads to good fish yield and annual production. Values recorded were higher than those reported by Abou *et al.* (2010) [2], but lower than those reported by Toko *et al.* (2007) [49] with *C. gariepinus* fed local agricultural by-products in earthen pond.

Economic analysis revealed that utilization of animal by-product such as fish viscera in diet for C. *gariepinus* lead to inexpensive diets costs. The replacement of FM by MFV in diet reduced feed costs per kilogram to 0.46 US$ kg^{-1} and 0.44 US$ kg^{-1} for D30 and D50, respectively compared with coppens cost (2.20 US$ kg^{-1}). This reduction in cost diet reduces significantly the cost of kilogram weight gain by 79.34 and 76.03%, respectively, thus increasing certainly the incomes of farmers.

Conclusion

Fish viscera used in this study had been properly processed and can be a good source of high quality protein for replacement of fish meal in *C. gariepinus* diet. Fish meal can be fully replaced by marine fish viscera without any adverse effect on growth performance. However, the results of this study suggest using the MFV up to 30% in diet for viable aquaculture of C. *gariepinus* in Benin rural areas.

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