

Effect of Fish Meal Replacement by Unconventional Meal in the Post-Larval Diet of *Clarias Gariepinus* in Benin (West Africa)

Edmond Sossoukpe*, Isidore N. Odjo, Théophile Godome, Emile D. Fiogbe

Laboratory of Wetland Research, Department of Zoology, Faculty of Sciences and Techniques, University of Abomey-Calavi, Abomey-Calavi, Benin

Email: *edmondsossoukpe67@gmail.com, odjoisidore@gmail.com, theogod@gmail.com, edfiogbe@yahoo.f

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Abstract

To reduce the pressure on aquatic resources due to the use of fishmeal in fish feed, the mastery and use of alternative sources of fishmeal in all stages of fish development are essential. Five diets including 4 experimental and one control diet were considered. Experimental diets of post-larvae were developed from unconventional ingredients in which fishmeal was completely replaced by a mixture of brewer's yeast, chicken viscera and maggots. These foods were tested on *Clarias gariepinus* post-larvae with an initial average weight of 80 ± 1 mg. 1500 fish individuals were equally distributed in 15 concrete basins with a volume of 500 L forming five treatments in triplicate. Fish individuals were fed, manually at a ration rate of 20%, four times per day. The specific growth rates were 8.38%/day in imported food and 6.35%/day in the experimental food receiving 45.5% of protein from unconventional meal. Similar results were obtained with the feed consumption index. These encouraging results show that it is possible to partially or completely replace fishmeal in catfish feed and obtain an economically profitable production. The recovery of waste such as chicken viscera, brewer's yeast and soybean meal in fish food formulation as tested in this study is a good contribution to environmental sanitation.

Keywords

Maggot Meal, Brewer's Yeast, Diet, Post-Larvae, Fish Meal Replacement, Benin

1. Introduction

In intensive aquaculture, the feed station accounts for a large part of the cost of

producing fish. The economic interest of this type of farming is therefore highly dependent on the availability and cost of food (Djissou *et al.*, 2016 [1]; Anvo *et al.*, 2016 [2]). Thus, the reduction of food-related costs, and consequently the control of the production cost of farmed fish, is one of the priorities in aquaculture (Djissou *et al.*, 2017 [3]; Odjo *et al.*, 2018 [4]). Fishmeal is generally the major component of food in aquaculture. Indeed, it is rich in Essential Amino Acids (AAE) whose profile corresponds remarkably to the needs of fish (Azaza *et al.* 2005 [5], Medale *et al.*, 2013 [6]). This conventional protein source accounts for 40% - 60% of the total protein in standard feeds for catfish (Azaza *et al.*, 2005 [5]). Its high purchase price and the irregularity of its quality have directed research towards alternative sources of proteins, animal and vegetable, which are not directly usable for human consumption (Djissou *et al.*, 2016 [1]; Medale *et al.*, 2013 [6]; Shiao *et al.*, 1989 [7]; El-Sayed, 1990 [8]; Imorou Toko *et al.*, 2007 [9]).

In order to limit the negative effects of each alternative raw material on fishmeal, the strategy adopted was to replace fishmeal with a mixture of vegetable and animal protein sources ([3]) and then to pay particular attention to the aspects identified as limiting: palatability of the food, digestibility of its components, nitrogen intake, in particular amino acid composition and energy content. For example, experimental trials have demonstrated the possibility of replacing 75% - 90% of fishmeal with vegetable and/or animal protein sources in Tilapia foods (Azaza *et al.*, 2006 [10]; Djissou *et al.*, 2017 [3]; Djissou *et al.*, 2019 [11]), African catfish (Sogesan *et al.* (2008) [12]; Djissou *et al.* (2016) [13]), European bass ([14]), sea bream (Sitjà-Bobadilla *et al.* [15]), Atlantic salmon (Epse *et al.* (2006) [16]), rainbow trout (Kaushik *et al.* (1995) [17]; Palti *et al.* (2006) [18]).

In Benin, the supply of fishery resources is proving difficult on an ongoing basis. This situation is the result of irrational exploitation of these resources which has led to a considerable reduction of fish products giving way to small species and the rarity of large species such as *Clarias gariepinus*, *Heterobranchus longifilis*, *Heterotis niloticus*, etc. (Lalèyè (1997) [19]; El-Sayed (1990) [8]). In 2016, the needs were estimated at more than 144,247 tonnes, while national fish production amounted to only 43,695 tonnes (Directorate of Fisheries Production, 2016 [20]). Beninese fisheries cannot cover domestic demand. Thus, over 45,000 tonnes of fishery products have been imported in recent years. In this circumstance, aquaculture appears to be the most important solution that can fill the strong demand for fish for Beninese food. But the development of aquaculture remains linked to the availability of food, quality and low cost, which is a key factor for the success of any livestock (Agadjohouédé *et al.* (2011) [21]). To do this, protein sources of high animal biological value maggot, chicken viscera and vegetable brewer's yeast (Djissou *et al.* (2016) [1]) and Odjo *et al.* (2018) [4]) have been used to formulate and produce quality foods (nutritional requirements) without fish meal to feed *Clarias gariepinus* post-larvae. This study aims

to completely replace fish meal with a mixture of local sources of protein in the post-larval diet of *Clarias gariepinus*.

2. Material and Methods

2.1. Experimental Procedure

This study was carried out in basins installed in open circuit at the experimental fish breeding station of the Laboratory of Wetlands Research of the Faculty of Sciences and Technologies of the University of Abomey-Calavi (Benin). Five diets including 4 experimental D2, D3, D4 and D5 and a control diet D1 (commercial food), all isoproteic ($45.1\% \pm 0.4\%$) and isoenergetic ($19.08 \pm 0.35 \text{ kJ}\cdot\text{g}^{-1}$) were tested. Experimental diets used in post-larval African catfish were developed from unconventional ingredients (**Table 1**). Within these diets, fish meal was completely replaced by a mixture of brewer's yeast, chicken viscera and maggots (**Table 2**).

The raw ingredients are finely ground and sieved using a 400 micron sieve. For each food, the ingredients were weighed and mixed until a homogeneous powder was added to which vegetable oil and CMV (vitamin-mineral complex) were added. Water was then added at a rate of 50% dry matter (Djissou, 2017 [22]), so as to obtain a malleable paste which, passed through a chopper (Moulinex HV8), gives filaments of 1.2 mm diameter (spaghetti). These filaments are subsequently dried in the sun, broken to the desired size, bagged and stored until dispensed. These foods were tested on post-larvae of *Clarias gariepinus*, with an initial average weight of $80 \pm 1 \text{ mg}$. The 1500 fish individuals were weighed and randomly distributed in 15 concrete ground circular basins with a volume of 500 L of useful volume, *i.e.* 100 fish per pool, thus forming five triplicate treatments each corresponding to a food item.

The fish individuals are stored in the basins 5 days before the beginning of the experiment to acclimatize them to the new conditions. The basins are fed with drilling water with a temperature of $27.6^\circ\text{C} \pm 3.1^\circ\text{C}$ and a flow rate of 3 L/min, ensuring oxygen content greater than 70% of the saturation. The pH, the dissolved oxygen ($\text{mg}\cdot\text{L}^{-1}$) and the electrical conductivity of the water ($\mu\text{s}/\text{cm}$) were measured every 48 h, in the morning at 7 h and in the evening at 18 h. Fish individuals are fed, manually, with the experimental feed at a ration rate of 20%, at four meals per day (8:00 am, 11:00 am, 2:00 pm and 5:00 pm). Every 05 days, fishing control is done and fish individuals are weighed and counted to adjust the new ration.

Table 1. Biochemical composition of the ingredients (expressed in % of the material).

Ingredients	Dry matter	Protein	Lipid	Ash
Brewer's yeast	94.3	50	3.7	13.18
Chicken Viscera	96.31	71.8	18.7	3.8
Maggot	92	54.6	15	10.95

Table 2. Biochemical composition, formulation and nutritional value of diets for pre-enlarged *Clarias gariepinus* post-larvae.

Ingredients (%)	Diet				
	D ₁	D ₂	D ₃	D ₄	D ₅
Gross lipid	11.6	12.63	12.92	13.28	13.93
Raw fiber	0.23	1.01	2.26	3.09	4.24
Moisture	7.35	7.67	8.53	8.04	8.76
Chitin	-	0.95	1.90	2.86	3.81
Ash	10.82	9.95	9.14	7.63	6.2
Gross energy	18.73	18.96	18.92	19.08	19.43
Crude protein	45.0	44.8	45.0	45.3	45.5
Soybean meal	-	15.0	15.0	15.0	15.0
Brewer's yeast	-	15.0	16.0	17.0	18.0
Chicken Viscera	-	15.0	16.0	17.0	18.0
Cotton cakes	-	7.0	7.0	7.0	7.0
Maggot meal	-	30.0	28.0	26.0	24.0
Maize bran	-	10.0	10.0	10.0	10.0
Palm oil	-	5.0	5.0	5.0	5.0
Vitamin mix	-	1.0	1.0	1.0	1.0
Minéral mix	-	1.0	1.0	1.0	1.0
Methionine	-	1.0	1.0	1.0	1.0
<i>Nutritional value</i>					

2.2. Biochemical Analyses

For the experimental foods and the fish carcasses, the biochemical analyses were carried out in triplicate with the experimental foods, the homogenized carcasses of 30 fish individuals randomly sampled at the beginning of the experiment. These analyses were also performed on the homogenized carcasses of 10 fish individuals randomly sampled after days from the end of the experiment in each of the 15 experimental ponds, *i.e.* 30 fish individuals per treatment. Following analyses were carried out:

- Dry matter by drying in an oven at 105°C for 24 hours (AOAC, 1990);
- Crude ash content: after incineration in the oven at 550°C for 24 hours;
- Crude protein content: according to Kjeldahl method (Nx6.25) which is based on organic nitrogen mineralization into ammonia, using concentrated sulfuric acid (H₂SO₄);
- Gross energy content: An adiabatic bomb calorimeter was used for all measurements and was calibrated with benzoic acid BE measurements were performed in duplicate with a difference of less than 30 kcal/kg M. on the other

hand, the gross energy of the commercial food which is the witness was obtained in the literature.

- Crude lipid content. Crude lipids were extracted by hot method using a mixture of chloroform (methanol 2:1 v/v);
- Fibers were determined by the method described by Anvo *et al.* (2016) [2]) and unlike chitin was measured using (Stelmock *et al.* (1985) [23]) method.

2.3. Growth Performance and Feed Efficiency

Nutrient growth and utilization parameters were calculated for each treatment as follows:

$$\text{Average Daily Gain (ADG)} (\text{mg/day}) = \frac{\text{weight gain (WG)}}{\text{Duration}}$$

$$\begin{aligned} \text{Specific Growth Rate (SGR\%/day)} \\ = \frac{\ln(\text{Final average weight}) - \ln(\text{initial average weight})}{\text{Number of days}} \times 100 \end{aligned}$$

$$\text{Consumption Index (CI)} = \frac{\text{food intake}}{\text{weight gain}}$$

$$\text{Cannibalism Rate (CR)} (\%) = \frac{\text{number of missing larvae}}{\text{initial number of larvae}} \times 100$$

2.4. Statistical Analyses

The biometric data for each repetition are considered as an observation. These results were compared statistically by the one-way analysis of variance (ANOVA) using the Statviews software (version 5.01) after prior checking of the homogeneity of the variances and the normality of the data to be analyzed. When ANOVA was significant, Fisher's LSD test was used for the multiple comparisons of averages; 5% level of significance is retained for these comparisons.

3. Results and Discussion

3.1. Trend in Growth Rhythm of Fish Biomass

Figure 1 shows changes in post-larvae biomass as function of diet and feeding period. An increase in the biomass of the post-larvae as function of time is noted with each of the five diets. The best biomasses at the end of the experiment were obtained with diets D1 (1.07 ± 0.14 g) and D5 (0.58 ± 0.10 g) respectively while the lowest biomass was obtained with diet D3 (0.34 ± 0.13 g).

These results show that the feed formulated with fishmeal provides farmed fish with the best amino acids essential for good growth. The results obtained with the replacement of this source of protein by unconventional sources which provide 45.5% of protein in the diet are quite encouraging and show that it is possible to substitute fishmeal with other ingredients.

The crude protein content of the tested diets in this study is slightly higher

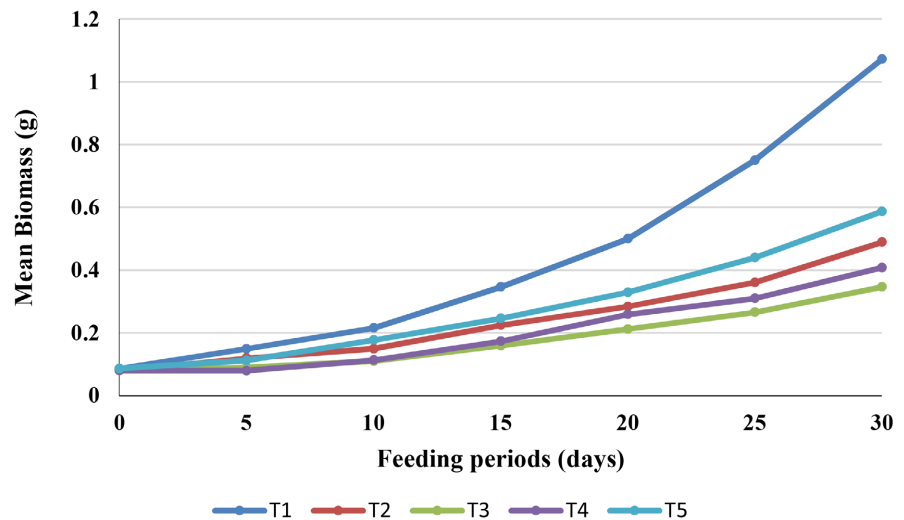


Figure 1. Evolution in post-larvae biomass as function of diet and feeding periods.

than the range of optimum required for catfish (*Clarias gariepinus*, *Heterobranchus bidorsalis* and *Heteroclaris*) which is between 40% and 42.5% (Reidel, 2007 [24]; Fagbenro *et al.*, 1992 [25], Eyo, 1996 [26], Monebi et Ugwumba, 2013 [27]).

Several studies have shown that when fish meal is completely replaced by maggot meal in fish feed, the growth of fish is reduced as in *Heterobranchus longifilis* (Sogbesan *et al.*, 2007 [28]), *Heteroclaris* (Monebi and Ugwumba, 2013 [27]), *Clarias anguillaris* (Madu and Ufodike, 2003 [29]) and *Clarias gariepinus* (Oyelese, 2007 [30]). In fact, in the use of alternative sources of high nutrient fish meal (digestibility and nutritional quality) in fish feed, the identified protein sources must, in addition to digestibility, have a good biological value and a good amino acid content especially essential amino acid (Djissou, 2017 [22]).

In this study, the alternative sources used (maggot + brewer's yeast + chicken viscera) in combination for the total replacement of fishmeal, are good protein sources with a good crude protein content (50% - 71.8%) and in essential amino acids, a better coefficient of predictive protein efficiency, a high chemical index and a very high digestibility (>90%) (Djissou *et al.*, 2018 [31]). According to Médale and Kaushik (2009) [32], the proteins provided by the diet must allow fish individuals to cover their nitrogen requirements for growth in both quantitative and qualitative terms.

3.2. Growth Performance and Feed Efficiency

The data obtained at the end of the experiment (Table 3) show that the final average fish weights vary between 692 ± 0.13 mg for the batch fed with the diet D_3 and 1170 ± 1.32 mg for the recipient fed with the control diet D_1 . The Fisher LSD test shows that there is no significant difference ($P > 0.05$) for the final average weight of diets D_2 , D_4 and D_5 with 751 mg, 829 mg and 930 mg respectively.

Table 3. Growth performance and feed utilization of fish fed experimental diets.

Parameters	D ₁	D ₂	D ₃	D ₄	D ₅
IW (mg)	80 ± 1 ^a	80 ± 1 ^a	80 ± 1 ^a	80 ± 1 ^a	80 ± 1 ^a
FW (mg)	1170 ± 14 ^a	829 ± 6 ^{bc}	692 ± 13 ^c	751 ± 3 ^{bc}	930 ± 10 ^b
SGR (%/day)	8.38 ± 0.52 ^a	6.01 ± 0.46 ^{bc}	4.70 ± 1.33 ^c	5.42 ± 0.29 ^{bc}	6.35 ± 0.53 ^b
CI	0.57 ± 0.12 ^b	0.63 ± 0.10 ^b	0.9 ± 0.11 ^a	0.77 ± 0.08 ^{ab}	0.61 ± 0.14 ^b
SR (%)	65.33 ± 5.77 ^b	74 ± 4 ^a	64 ± 22.71 ^a	63.33 ± 18.14 ^a	66.66 ± 16.28 ^a
DWG (mg/day)	43.6 ± 3.3 ^a	29.96 ± 0.5 ^{ab}	24.48 ± 1.0 ^c	26.84 ± 1.4 ^{bc}	34 ± 2.6 ^a
CR	10.66 ± 2.26 ^a	11.33 ± 1.16 ^a	14 ± 3.28 ^a	12.33 ± 2.02 ^a	10.33 ± 1.37 ^a

^{abc}Values on the same line and not having the same letter are significantly different ($P < 0.05$).

Calculated SGRs were 8.38%/day in D₁ and 6.35%/day in D₅ with a significant difference between D₁ and each of the experimental diets (D₂, D₃, D₄, D₅). The consumption indices were range between 0.57 and 0.90, respectively for diets D₁ and D₃. Thus, the batch receiving the feed containing the fish meal (D₁-commercial food), and more particularly the D₂ and D₅ batches, are distinguished by a better weight growth and food processing. This performance is more pronounced in batch D₅, receiving a 45.5% crude protein feed, with a Specific Growth Rate (SGR) of 6.35% and a Consumption Index (CI) of 0.61.

The results of this study show that the commercial food (Coppens) gave the best growth and food utilization performance. D₅ (formulated with 18% LB, 18% CV and 24% maggots) gave the closest result to what is recorded in post-larvae fed with Coppens. The specific growth rates (SGR) obtained with D₁ and D₅ were significantly close to the value of 17.3%/day observed in *C. gariepinus* larvae fed with *Artemia salina* nauplii for 25 days (Olsen *et al.*, 2006 [33]). Anvo (2017) [34] had reported a SGR value of 22.87%/in larvae fed with a mixture (*Artemia* and a dry food) while Ward and Reichert (1986) [35] had obtained a SGR value of 11.28%/day and 14.3%/day in larvae fed respectively with zooplankton and *Artemia salina*. The differences observed in these specific growth rates can be explained by the specifically quality content of the various components (proteins, lipids, amino acids and fatty acids, minerals...) of the distributed diets and certain factors such as temperature, stocking density and duration of rearing (Longvah *et al.*, 2011 [36]).

The analysis of zootechnical performance features and food utilization in post-larvae of *C. gariepinus* is influenced by distributed diets. In fact, the best growth and food utilization performances observed in post-larvae fed with D₁ are due firstly to fishmeal, which is a complete ingredient containing nutrients that promote growth, by the buoyancy of the extruded commercial food compared to the formulated diets (D₂ to D₅). Buoyancy is one of the physical properties of fish feed favoring dietary intake. Thus, the decrease in food intake asso-

ciated with the incorporation of a mixture of unconventional sources of protein in diets could explain the growth reduction observed. Indeed, the increase in the amount of chitin in diets (D₂ to D₅) which is a consequence of the increase in the level of incorporation of alternative sources to fishmeal in diets could result in low consumption and high ICA (Achionye-Nzeh, 2012 [37]). Chitin (a fiber) could bind nutrients such as fat, protein and minerals (Longvah *et al.*, 2011 [36], Faruque *et al.*, 2010 [38]). It can also interfere with the use of these nutrients and reduce their bioavailability (Agadjihouédé *et al.*, 2012 [39]).

Lot of post-larvae fed experimental diets did not show a significant difference in cannibalism rates. These results can be explained by size uniformity of post-larvae that may significantly reduce cannibalism (Baras *et al.*, 1999 [40]).

In total, it is possible to completely replace fishmeal with other sources of animal protein; which is not the case for proteins of vegetable origin. Indeed, Muyinda *et al.* (2021) [41] who tested *Rastrineobola argetea* and Roasted Soybean meal as protein ingredients for brood stock African Catfish (*Clarias gariepinus*) in Uganda concluded that it is implausible to substitute fishmeal 100% by soybean meal even with treatment to remove ant nutritional factors.

3.3. Biochemical Composition of Fish Carcasses Fed with Experimental Diets

Regarding the composition of the carcass (Table 4), the analyses showed significant difference between the protein content of the fish harvested at the beginning of the experiment and those fed with the 5 diets. For the lipid contents, the results showed that the fish individuals are fatter at the end than at the beginning of the experiment with the four tested diets (D₂ - D₅). In addition, statistical analysis shows that the lipid content increases significantly with the incorporation rate of chicken viscera. For the ash and dry matter contents, it varies more or less in the carcasses of the fish at the end of the experiment compared to the initial state.

4. Conclusion

It appears from the experiments carried out that the total replacement of fish meal by a combination of chicken viscera, maggot meal and brewer's yeast is

Table 4. Percentage composition of homogenized carcasses of fish fed experimental diets.

Parameters	Initial	D ₁	D ₂	D ₃	D ₄	D ₅
Protein	10.88 ^a	14.32 ± 0.23 ^b	13.79 ± 0.39 ^b	13.12 ± 0.18 ^c	12.93 ± 0.11 ^c	12.80 ± 0.22 ^c
Lipid	3.67 ^a	7.28 ± 0.11 ^b	7.77 ± 0.08 ^{bc}	8.03 ± 0.12 ^{cd}	8.15 ± 0.09 ^{cd}	8.51 ± 0.13 ^d
Ash	2.58 ^a	3.53 ± 0.05 ^b	3.21 ± 0.02 ^{bc}	2.86 ± 0.07 ^{acd}	2.71 ± 0.06 ^{acd}	2.44 ± 0.06 ^{ad}
Dry matter	85.33 ^a	84.1 ± 1.91 ^a	87.7 ± 1.67 ^a	84.3 ± 1.29 ^a	79.1 ± 1.78 ^b	85.6 ± 1.12 ^a

^{abc}Values on the same line and not having the same letter are significantly different (P < 0.05).

possible. The food formulated from these three ingredients with a protein rate of 45.5% has given very encouraging results. The specific growth rate of fish individuals fed with this experimental food is close to that of those fed with the imported food. It is therefore possible to completely replace fishmeal in catfish feed and obtain an economically profitable production. Finally, the recovery of waste such as chicken viscera, brewer's yeast and soybean meal in the production of the food tested in this study is a good contribution to environmental sanitation.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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