Growth Performance and Survival of Clarias gariepinus Larvae Fed with Varying Inclusions of beef Brain Meal

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ABSTRACT

Clarias gariepinus culture remains undeveloped in Côte d’Ivoire due to the lack of locally quality feed, fingerlings, and the high cost of imported feed Artemia salina. Because of the importance of feed and feeding strategy in fish fingerling production, beef brain meal was investigated as alternative compounded feed. Feeding trial was completed with five isonitrogenous diets at 35% crude protein formulated by substituting Artemia salina for beef brain (BB) meal on the basis of crude protein at 0% (Control diet), 25% (BB35), 50% (BB50), 75% (BB75) and 100% (BB100) level. Catfish, Clarias gariepinus larvae with an average weight of 0.006 ± 0.001 g were fed with experiment diets in three replicate groups, three times a day (07:00, 12:00 and 17:00 hours) ad libitum 49 days. At the end of rearing period, highest values of growth parameters were recorded from larvae fed with BB100, BB75, and BB50. Beef brain meal increased the dietary total fat when the feed conversion ratio decreased. Fish fed diets BB25 and BB35 recorded the highest values of survival rate. Studies on the best feeding rates and water quality monitoring should be carried out to improve survival of Clarias gariepinus larvae fed with high level of beef brain meal in diet.

1. INTRODUCTION

Culture of African catfish, Clarias gariepinus Burchell 1822 has received considerable attention since the early 1970 - 1980s [1]. This catfish has been considered a fish of great promise for fish farming in Africa because of its high growth rate, resistance, and appreciation in a wide number of African countries. Clarias gariepinus has become a popular species for aquaculture in sub-Saharan Africa particularly in Nigeria where it represents the most aquaculture produced catfish [2]. However, Clarias gariepinus culture remains undeveloped in Côte d’Ivoire [3, 4]. The production of Clarias gariepinus and catfish generally faces lack of capital, high cost of imported quality feed, lack of locally quality feed, little presence of hatcheries producing high quality seed, predation and cannibalism of larvae, and lack of fingerlings [5, 6, 4]. Therefore, fingerling production is one of the many challenges for successful Clarias gariepinus culture in Côte d’Ivoire. Feeds and feeding of the larvae and fry of the catfishes influence the growth and survival of the fish [7, 8]. Starter feeds play a key role in the growth of larvae. They provide the required nutrients (35-40% proteins), amino acids and fatty acids to promote optimal larval growth and health [9]. Artemia nauplii and decapsulated cysts are used with successful outcomes in feeding most larvae and fry of C. gariepinus [10, 11, 12, 13].

Artemia salina was used as the main protein source due to its high proteins, balanced amino acids profile, essential fatty acids, minerals and vitamins content, palatability, and highly digestibility [14].

In addition, Artemia cysts can be stored in cans during a longer period and used after 24h of incubation, making them the most convenient and available starter feed for Aquaculture [15]. However the increasing cost of Artemia cysts is a major constraint to most fish farmers especially in West Africa. Therefore, it is of main importance to carry out more studies on alternative feeds. Substitution of imported Artemia salina by locally compounded diets is essential for lowering production cost while promoting quality catfish fingerlings production. Also various conventional and non-conventional animal by-products have been tested to substitute Artemia salina in catfish compounded starter feed [16, 15, 17, 18].
In these feeding trials, the diets were formulated with roquette seed meal, rotifer Brachionus calyciflorus, fermented fish silage, probiotic Lactobacillus acidophilus, beef brain, beef brain meal, maggot meal, fish meal, blood meal, and soybean meal [15, 16, 17]. Ossey et al. [19, 20] reported that the use of beef brain meal (36.5 % proteins; 32% lipid) fortified with iron, chlorine, phosphorus, catfish premix vitamins/aminos-acids, lysine and methionine in formulated diet at 35% proteins content improves beef brain meal potentials in Heterobranchus longifilis larvae feeding. Sheep beef brain is locally available, and the production process of fortified beef brain meal is simple. Ossey et al. [19, 20] have reported good growth and feed utilization results using beef brain meal in Heterobranchus longifilis larvae. However, beef brain meal has not yet been used to substitute Artemia salina in Clarias gariepinus larvae diets. The aim of this study was to determine the optimal level of replacement of Artemia salina by beef brain meal in the diet of Clarias gariepinus larvae. The growth trial responses may help to reduce C. gariepinus fingerling production cost and increase fingerlings availability for improving Côte d’Ivoire aquaculture catfish production.

2. MATERIALS AND METHODS

2.1 Experimental diets
Artemia salina, beef brain meal, maize flour, Maridav (imported enriched soy bean meal) were used as main raw materials to formulate diets in this study. Palm oil, Lysin, methionin, and VITAMYNOLITE super premix were added to the different mix of raw materials. Artemia salina was bought from a pharmacy shop at Abidjan, Ivory Coast at 210.57 USD kg \(^{-1}\) and hatched followed by Slembrouch and Legendre [21] to obtain 1.5 kg of Artemia salina meal. Beef brain was purchased from local market in Abidjan at 2.32 – 2.65 USD kg \(^{-1}\), then, 4.5-5.5 kg of beef brain were dried at 80°C for 24h and crushed into powder to obtained 1kg of beef brain meal (9.28 – 14.58 USD kg \(^{-1}\)) as described by Ossey et al. [19]. Maize flour, Maridav (imported enriched Soy bean meal), palm oil, vitamins and amino acids were obtained from the local animal feeds sellers. Artemia salina (49.0% crude proteins), enriched soy bean meal (44.8% crude proteins) maize flour (09.50% crude proteins), palm oil (2%), Lysine (2.13%), Methionine (1.61%) and VITAMYNOLYTE super premix (2%) were used for balanced control diet formulation at 35% proteins (Table 1).

Then, five isonitrogenous diets at 35% crude proteins were formulated by substituting Artemia salina by beef brain meal (36.50% crude proteins) on the basis of crude proteins as follows: Control diet = 0% beef brain proteins replaced Artemia salina proteins; (BB\(\_1\)) = 25% beef brain proteins replaced Artemia salina proteins; (BB\(\_2\)) = 50% beef brain proteins replaced Artemia salina proteins; (BB\(\_3\)) = 75% beef brain proteins replaced Artemia salina proteins and (BB\(\_4\)) = 100% beef brain proteins replaced Artemia salina proteins. All diets were formulated using linear programming method as described by Koumi [22]. Ingredients and chemical composition of the control and experiment diets are presented in Table 1.

For each formula, all the ingredients were weighted and ground using a homogenous mixture grinder. Warm water at 80°C (800 ML kg \(^{-1}\)) was added and mixed. The paste obtained was dried in electric oven at 60°C for 48 hours. The dried paste was crushed into powder with pestle and mortar to obtained meat powder used in electric oven at 60°C for 48 hours. The dried paste was crushed into powder with pestle and mortar to obtained meat powder used in electric oven at 60°C for 48 hours. The dried paste was crushed into powder with pestle and mortar to obtained meat powder used in electric oven at 60°C for 48 hours. The dried paste was crushed into powder with pestle and mortar to obtained meat powder used in electric oven at 60°C for 48 hours.

Table 1: Formulation (%) and proximate composition of the diets.

| Ingredients composition (%) | Control diet (0%) | BB\(\_1\) (25 %) | BB\(\_2\) (50 %) | BB\(\_3\) (75 %) | BB\(\_4\) (100 %) |
|----------------------------|------------------|-----------------|-----------------|-----------------|-----------------|
| Artemia meal               | 35.80            | 44.20           | 50.00           | 57.00           | 61.50           |
| Beef brain meal            | 0.00             | 20.00           | 30.00           | 50.00           | 60.00           |
| Maridav                    | 1.00             | 10.00           | 0.00            | 10.00           | 10.00           |
| Maize flour                | 24.56            | 18.00           | 10.76           | 5.57            | 1.77            |
| Palm oil                   | 0.02             | 2.00            | 2.00            | 2.00            | 2.00            |
| Lysine                     | 2.13             | 2.13            | 2.13            | 2.13            | 2.13            |
| Methionine                 | 2.04             | 2.04            | 2.04            | 2.04            | 2.04            |
| VITAMYNOLYTE Super prémix  | 5.77             | 5.77            | 5.77            | 5.77            | 5.77            |

| Proximate analysis          |                  |                  |                  |                  |                  |
|----------------------------|------------------|-----------------|-----------------|-----------------|-----------------|
| Moisture (%)                | 10.60            | 10.88           | 11.08           | 11.18           | 11.18           |
| Crude proteins (% DM)       | 35.13            | 35.16           | 35.34           | 35.97           | 35.97           |
| Total fat (% DM)            | 04.76            | 14.54           | 24.99           | 34.84           | 46.10           |
| Ash (% DM)                  | 03.21            | 03.12           | 03.03           | 02.93           | 02.89           |
| Crude fiber (% DM)          | 03.90            | 03.03           | 02.11           | 01.22           | 0.33            |
| Nitrogen free extract (NFE) (%DM)\(^{1}\) | 42.40           | 33.27           | 23.45           | 14.86           | 07.27           |
| Gross energy (kJ g \(^{-1}\)) | 16.94           | 19.18           | 21.60           | 23.87           | 27.04           |
| P/E (mg kg \(^{-1}\))\(^{1}\) | 20.73           | 18.33           | 16.36           | 14.65           | 13.09           |

\(^{1}\)Composition for 1 kg of Maridav; Crude Protein = 44.8%; Fat = 3, 5%; Ash =12, 1%; Moisture = 9, 5%; Crude fiber = 02, 9%; Lysine = 03, 2%; Methionine = 01, 2%; Methionine + Cysteine = 01, 8%; Calcium = 02, 2%; Phosphorus = 01, 0%; Sodium = 0, 5%; Vitamins A = 46,000 IU; Vitamins D3 = 09, 590 IU; Vitamins E = 63 mg; Vitamins K3 = 6 mg; Vitamins B1 = 4 mg; Vitamins B2 = 20,000 mg; Niacin = 15,000 mg; Pantothenic acid = 35,000 mg; Vitamins B6 = 08,000 mg; Vitamins B12 = 97,000 mg; Biotin = 194,000 mg; Folic acid = 2,000 mg; Choline chloride = 807,00 mg; Iron = 496,00 mg; Copper = 401,00 mg; Zinc = 232,00 mg; Manganese = 341,00 mg; Iodine = 04,00 mg; Selenium = 0,60 mg; Salinomycin = 226,00 ppm

\(^{2}\)Composition for 1 kg of premix; Vitamins A = 12,00 M UI; Vitamins D3 = 03,20 M UI; Vitamins E = 2500 mg; Vitamins K3 = 4000 mg; Vitamins B1 = 5000 mg; Vitamins B2 = 500 mg; Vitamins B6 = 2500 mg; Vitamins B12 = 5 mg; Biotin = 5 mg; Folic acid = 250 mg; Copper = 401,00 mg; Zinc = 232,00 mg; Manganese = 341,00 mg; Iodine = 04,00 mg; Selenium = 0,60 mg; Salinomycin = 226,00 ppm

\(^{3}\)Nitrogen free extract (NFE) = 100; (% protein + % lipid + % moisture + % ash + % fiber)

\(^{4}\)Gross Energy = protein × 22.20 kJ/g + lipid × 38.90 kJ/g + Nitrogen free extract × 17, 20 kJ/g; P/E = Protein to energy ratio in mg protein/Kj gross energy.
2.2 Experimental fish and feeding trial

Total of 750 catfish *Clarias gariepinus* larvae with average weight 0.006 ± 0.001g were used. The larvae used in this experiment were obtained from of artificial reproduction following Legendre [23] at Centre de Recherches Oceanologiques (CRO), Côte d’Ivoire. Three days-age larvae were transferred in aquarium (39.4 cm × 50.2 cm × 27 cm), capacity of 50 L and acclimated in four days prior to the beginning of the growth trial. Three replicate were constituted by diet and the feeding trial was conducted in 15 aquariums in CRO hatchery. Fish were weighted counted and stored at density of 1 larva L⁻¹ in each aquarium. All the larvae were fed experimental diets three times daily (07:00, 12:00 and 17:00 hours) *ad libitum* during 49 days. Every day, dead fish of each aquarium were removed and counted. Once a week, fifteen larvae were randomly sampled in each aquarium to measure total weight using electronic balance SARTORIUS L 6200 S (accuracy of 0.001 g) and the total length (L) was measured to the nearest half millimeter.

Subsequently, all larvae were weight and ration was adjusted to reflect the new weight. At the end of rearing, all survival fish were collected, weighted, and counted from each aquarium and individual total length and body wet weight were also recorded by fish. A distinction was draw between cannibalism and natural death. Missing fish were presumed to have succumbed to a complete cannibalism [24]. Natural death was determined by the presence of complete fish floating in the aquarium [25].

2.3 Growth parameters and nutrient utilization

The growth indices and nutrient utilization parameters were calculated for each treatment as follows: Weight gain (WG) (g) = final body weight – initial body weight; Daily weight gain (DWG) (g day⁻¹) = (final body weight – initial body weight)/number of day; Specific growth rate (SGR) (% day⁻¹) = [ln (final body weight) – ln (initial body weight)]×100/ number of day; Feed conversion ratio (FCR) = total weight of feed consumed (g)/ wet biomass gain (g); Total weight of feed consumed is obtained by total feed distributed fewer uneaten food; Survival rate (SR) (%) = (final number of larvae/initial number of larvae)×100; Cannibalism rate (CR) (%) = (number of larvae missing + incomplete dead larvae/initial number of larvae)×100; Mortality rate (MR) (%) = (number of complete dead larvae/initial number of larvae)×100.

2.4 Biochemical analysis

The proximate composition of experimental diats was determined according to AOAC [26] methods. Dry matter (DM) was determined after oven drying at 105°C for 24 hours until constant weight; crude protein (% N x 6.25) was determined using kjeldahl method; crude lipid by Soxhlet extraction with hexane; Ash was measured by incineration at 550°C in a muffle furnace for 24 hours; crude fibre were measured by acid digestion following by ashing the dry residue at 550°C in a muffle furnace for 4 h, while nitrogen-free extract (NFE) was calculated by difference. The gross energy contents of the diets were calculated on the basis of their crude proteins, lipid and carbohydrate contents using the energy equivalents of 22.2, 38.9 and 17.15 kJ g⁻¹ respectively [27].

2.5 Statistical Analysis

Data analysis was performed using Statistica 7.1 software. Data were presented as mean ± standard deviation (SD). Results were compared using one-way analysis of variance (ANOVA). The Tukey’s multiple range tests was used to compare differences among treatment means. Significant differences were considered at p < 0.05.

3. RESULTS

The growth performance parameters, nutrient utilization indices and survival rate of *Clarias gariepinus* fed Control diet and experimental diets BB₂₅, BB₅₀, BB₇₅ and BB₁₀₀ are shown in (Table 2).

3.1 Growth performance parameters

Final body weight (FBW), weight gain (WG), daily weight gain (DWG) and specific growth rate (SGR) were significantly (p < 0.05) affected by the levels of Artemia protein substitution by beef brain protein in control diet. The highest significant values of final body weight and weight gain were recorded from larvae fed BB₁₀₀ (FBW = 3.45 ± 0.02 g; WG = 3.44 ± 0.01 g) followed by fish fed BB₇₅ (FBW = 3.28 ± 0.03 g; WG = 3.27 ± 0.02 g) and BB₅₀ (FBW = 3.17 ± 0.02 g; WG = 3.16 ± 0.03 g) which were different significantly.

The lowest significant (p<0.05) values of these parameters were obtained in larvae fed BB₂₅ (FBW = 2.83 ± 0.10 g; WG = 2.82 ± 0.10 g) and control diet (2.19 ± 0.13 g; 2.18 ± 0.16 g). However, significant (p<0.05) highest values of daily weight gain and specific growth rate were recorded by fish fed BB₁₀₀ (0.07 ± 0.005 g day⁻¹; 12.83 ± 0.13 % day⁻¹), BB₇₅ (0.07 ± 0.004 g day⁻¹; 12.67 ± 0.15 % day⁻¹) and BB₅₀ (0.06 ± 0.005 g day⁻¹; 12.54 ± 0.14 % day⁻¹) which did not differ significantly (p<0.05). The significantly lowest values of these two parameters were observed in fish fed control diet.

3.2 Nutrient utilization indices

The different values of feed conversion ratio (FCR) recorded in growth trial were affected by the level of *Artemia salina* replacement by beef brain meal in control diet. Results showed that FCR values tended to decreased with beef brain meal inclusion level in control diet.

The significant (p<0.05) best value of feed conversion ratio was recorded from fish fed BB₁₀₀ (1.22 ± 0.04), BB₇₅ (1.33 ± 0.24) and BB₅₀ (1.43 ± 0.27) without significant difference followed by those of fish fed BB₂₅ (1.68 ± 0.12) and the lowest value of this parameter was obtained by fish fed control diet (1.88 ± 0.29).
Clarias gariepinus larvae with increasing dietary lipid level in diets containing 35% crude protein. Lipids are a group of natural organic compounds comprising fats, oils, phospholipids and sterols. Moreover fats have distinct advantage of being almost completely digestible. In fish ration, the neutral lipid components and the sources of lipids are useful elements for diet formulation and are especially essential in feed of fry and fingerlings, which require high energy intake for rapid growth [28, 29]. They are nonprotein calorie sources which are generally more digestible than some carbohydrates [30]. Dietary lipids influence flavor and texture of prepared feeds. They were utilized in fish as a major energy source to spare proteins, provide essential fatty acids needed for proper functioning of many physiological processes and maintenance of membrane fluidity and permeability as well as for growth and survival of fish [31, 32, 33]. In general, lipid requirement for catfish (Clarias gariepinus, Heterobranchus longifilis) ranges between 5-10%. However, Cahu [34] showed that the increase of catfish dietary lipid more than lipid requirement improves growth. Moreover, Lee and Sinnhuber [28] reported that catfish can use 20-30% of the dry diet ingredients as fat, provided that adequate amounts of choline, methionine and tocopheral are present in the ration. In this study, the higher lipid level more than catfish lipid requirement and the high values of gross energy present in the diets formulated at 35 % proteins was an advantage for good growth of larvae. This observation indicates good nutritional quality of the nutriments and biological values of feeds BB50, BB75 and BB100. In addition, the SGR values obtained with these diets (12.54-12.83 % day−1) were similar to the growth rates (12.03-12.73 % day−1) of larval and fry of Clarias gariepinus fed with Artemia salina and commercial high proteins (53.2-54%) diets [8]. These growth rates suggest that the tested diets BB50, BB75, and BB100 were suitable for rearing Clarias gariepinus catfish larvae.

The results also showed that relative highest cannibalism and mortality rates resulted in lowest survival rates for larvae fed with diets BB25 and BB100. During the experiment, water turbidity increased in the order diets BB25 < BB75 < BB100 in the aquarium because of the high lipid contents in feeds along with unconsumed feeds particles. Some studies have revealed that

### Table 2: Growth performance, nutrient utilization and survival rate of Clarias gariepinus larvae fed with the experimental diets.

| Parameters | Control diet (0%) | Experimental diets |
|------------|------------------|-------------------|
| Initial body weight (g) | 0.006 ± 0.001 | BB25 (25%) | BB50 (50%) | BB75 (75%) | BB100 (100%) |
| Final body weight (g) | 2.19 ± 0.13 | 0.006 ± 0.001 | 0.006 ± 0.001 | 0.006 ± 0.001 | 0.006 ± 0.001 |
| Weight gain (g) | 2.18 ± 0.16 | BB25 | BB50 | BB75 | BB100 |
| Daily weight gain (g day−1) | 0.04 ± 0.01 | 0.06 ± 0.010 | 0.06 ± 0.005 | 0.07 ± 0.004 | 0.07 ± 0.005 |
| Specific growth rate (% day−1) | 11.85 ± 0.05 | 12.37 ± 0.23 | 12.54 ± 0.14 | 12.67 ± 0.15 | 12.83 ± 0.13 |
| Initial biomass of fish (g) | 0.30±0.001 | 0.30±0.001 | 0.30±0.001 | 0.30±0.001 | 0.30±0.001 |
| Final biomass of fish (g) | 82.53±0.10 | 109.67±0.21 | 114.16±0.10 | 112.04±0.02 | 120.82±0.23 |
| Biomass gain (g) | 82.23±0.11 | 109.37±0.20 | 113.86±0.24 | 111.74±0.31 | 120.52±0.13 |
| Quantity of feed used (g) | 155.16±0.11 | 184.24±0.12 | 163.24±0.14 | 149.01±0.03 | 147.50±0.21 |
| Feed conversion ratio | 1.88 ± 0.29 | 1.68 ± 0.12 | 1.43 ± 0.27 | 1.33 ± 0.24 | 1.22 ± 0.04 |
| Cannibalism rate (%) | 23.32 ± 4.28 | 19.90 ± 0.97 | 21.85 ± 0.48 | 26.90 ± 0.29 | 29.66 ± 0.25 |
| Mortality rate (%) | 2.21 ± 0.09 | 1.35 ± 0.11 | 1.40 ± 0.20 | 1.75 ± 0.31 | 3.34 ± 0.03 |
| Survival rate (%) | 74.47 ± 0.46 | 78.75 ± 1.14 | 76.75 ± 0.25 | 71.35 ± 0.32 | 67.00 ± 0.35 |

Notes: a Mean values ± SD in the same row sharing the different superscript are significantly different (p < 0.05).
cannibalism and mortality are influenced by water quality management, fish density, feeding strategies, competition of food and stress [15, 25, 35]. In this study, larvae were stocked at low density (1 larva L\(^{-1}\)) and fed at libitum three times daily. Feeding fish at satiation could affect water quality and increase fish stress, mortality, and cannibalism. According to Faruque et al. [15], high feeding levels result in the highest mortality rate while low feeding levels might cause lower survival rate and growth. In order to minimize fish stress and offer the better environment for larvae growth, the best feeding rates of larvae that promote survival must be investigated with high lipid content feeds formulated with beef brain meal.

5. CONCLUSION

Growth trial responses showed that substitution of *Artemia salina* proteins for beef meal proteins in larvae *Clarias gariepinus* diets improves growth and feed efficiencies. The success of this substitution promotes locally available low cost quality feed for fingerling production. Studies on the best feeding rates and water quality monitoring should be carried out to improve survival of *Clarias gariepinus* larvae with beef brain diets. The utilization of high lipid beef brain diets for *Clarias gariepinus* larvae growth needs the determination of the best feeding rate, frequency and the monitoring of the water quality.

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