

Effects of Feeding by-Products; Maggot Meal, Fish Meal, Soybean Meal, Blood Meal and Beef Brain on Growth, Survival and Carcass Composition of African Catfish, *Heterobranchus Longifilis Valenciennes*, 1840 Larvae under Recirculating Conditions

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Abstract – A forty nine-days feeding trial was conducted in aquaria at density of 1 larval^L⁻¹ to evaluate the growth performances, survival, nutrient utilization and carcass composition of the African catfish *Heterobranchus longifilis* larvae fed trice daily *ad libitum* to triplicate groups with local by-products fish meal (FME), blood sausage of pig (BSA), soybean (SBE), beef brain (BBR), maggot flour (MFL) and *Artemia salina* (ASA) as reference diet (Experience 1). Diet had a significant effect on growth performance and carcass composition ($p < 0.05$). Final body weight, Final body length, Body weight gain, Specific growth rate and Coefficient of variation were highest in fish fed ASA and FME. Survival rate (SR) was highest in fish fed MFL. Coefficient of variation and cannibalism rate were highest in fish fed FME. Feed conversion ratio (3.38 ± 0.39 ; 5.30 ± 1.610) values were significantly ($p < 0.05$) lowest in fish fed MFL and ASA respectively. Inversely, Protein efficiency ratio (1.47 ± 0.20) was highest in fish fed BBR. For carcass composition, the highest carcass protein was obtained in fish fed ASA. Carcass crude lipid was highest in fish fed SBE. In the Experiment 2 three diets of Experiment 1 were selected by their growth and survival performances to prepare fortified diets. Thus soybean (SBE+), beef brain (BBR+) and maggot flour (MFL+) diets were fortified with 4 % of each mineral (iron, chlorine and phosphorus), 2 % premix vitamin/ amino-acid, and 1 % of each amino acid (lysine and methionine) and compared to *Artemia salina* as reference diet. Final body weight, Body weight gain and Specific growth rate were highest in fish fed MFL+. Inversely, Feed conversion rate (0.89 ± 0.30) was lowest in fish fed MFL+. Protein efficiency ratio (0.95 ± 0.04) was lowest in fish fed SBE+. Carcass composition showed significant difference ($p < 0.05$) among diets fortified. Carcass crude protein and ash contents were lowest in fish fed SBE+. In contrast, carcass crude lipid content was highest in this group.

Keywords – African Catfish *Heterobranchus Longifilis*, Larvae Nutrition, By-Products, Growth, Carcass Composition.

I. INTRODUCTION

The species *Heterobranchus longifilis* is one of the most suitable species for aquaculture in West Africa (Legendre, 1992; Otémé *et al.*, 1996). Because of its characteristics such as fast growth, tolerance to poor water quality, high fecundity, omnivorous feeding habit, and good acceptance

of commercial diet this species is a good candidate for aquaculture (Legendre, 1992; Fashina-Bombata and Busari, 2003). Despite of these advantages, one of the major constraints for successful rearing of this species remains the high mortality rate of larvae due to cannibalism injuries because of inadequate larva food and improper feeding regime (Hecht and Appelbaum, 1988; Otémé *et al.*, 1996).

African catfish accept a wide variety of agricultural by-products in the form of pelleted or dough feed (Medale and Kaushik, 2009; Pallabi *et al.*, 2013). Local plants supply a large proportion of the protein, vitamins and minerals to animals. Thus, significant effort has been directed toward evaluating the nutritive value of different nonconventional feed resources to formulate nutritionally balanced and cost effective diets for fish (Ray and Das, 1995). Feed is considered as the most critical input for augmenting fish production. In fact, proteins represent from 30 to 50 % of the feed ration of fish (Medale and Kaushik, 2009) and more than 60 % of the total production cost in fish farms (FAO, 1983). Because of the high cost of feed, majority of the fish farmers do not serve any more certain ingredients known as conventional in the formulation of fish feed (Eyo, 1995). Therefore, there is a great demand for scientifically developed fish feed in developing countries. It is generally advisable to make the use of local resources as fish feed as far as possible for farmers. In addition, these local resources enjoy high availability and accessibility in certain countries and are considerably cheaper than imported feeds such as *Artemia salina*, the feed reference for African catfish larvae feeding. Therefore, present study was undertaken to evaluate the effect of feeding by-products maggot meal, fish meal, soybean meal, blood meal and beef brain on growth, survival and carcass composition of *Heterobranchus longifilis* larvae.

II. MATERIAL AND METHODS

Experiment 1: Feeding with by-products

Five local by-products; fish meal, beef brain, soybean, blood sausage of pig and maggot flour were used as experimental diets for *Heterobranchus longifilis* larvae.

Fish meal, soybean, beef brain and blood were purchased from local markets in Abidjan. Pig blood sausage was prepared by cooking the scarlet ovine blood to obtain a paste. Soybean was dried at ambient temperature for 72 hours and was grounded into fine particles. Maggots were cultured from poultry droppings and fish carcass following the method of Mpoame *et al.* (2004). The maggots collected were weighed, blanched in hot water, oven dried at 70 °C for 24 h and crushed into powdery to obtain maggot meal. The *Artemia salina* cysts were incubated and hatched under optimal conditions as described by Slembrouck and Legendre (1988). The proximate composition of the experimental diets is shown in Table 1.

Experiment 2: Feeding with supplemented diets

Three diets of Experiment 1 were selected by their growth and survival performances to prepare fortified diets using minerals, vitamins and amino-acids premix in order to improve their potentials in larvae feeding. Thus, maggot-, brain- and soybean-diets were selected and 4 % of each mineral (iron, chlorine and phosphorus), 2 % premix vitamin/ amino-acid and 1 % of each amino acid (lysine and methionine) were added in each diet. The proximate composition of the experimental diets is shown in Table 2.

All the diets were packed in plastic containers and stored at -20°C until use. The *Artemia salina* nauplii were used as control diet in the two experiments.

Experimental fish and feeding trial

The African catfish *Heterobranchus longifilis* Valenciennes, 1840 larvae used in the two Experiments were obtained from induced brood stock reared in Layo Aquaculture station (5°19'N, 4°19'W; Ivory Cost). 3days-age larvae were transferred in aquaria (38.5 x 46.5 x 28 cm³), capacity of 50 L and acclimated in aquaria system for 4 d prior to the beginning of the growth trial. Water was supplied to each aquarium from 250 L head tank. Fish in each aquarium were weighted, counted and stored at density of 1 larva L⁻¹. Three replicate aquaria were constituted for each diet. During 49 days, fish were fed three times a day (08:00, 12:00 and 17:00 hours) *ad libitum*. A total of 900 and 600 larvae were used respectively in Experiment 1 and 2. Three times a week, undigested food particles and waste products were siphoned out with rubber hose before feeding fish. Once a week, 10 larvae were randomly sampled in each aquarium to measure growth (total length and wet weight). Total length was measured to the nearest half millimetre. Wet weight was measured on an electronic digital balance SARTORIUS L 6200 S (accuracy of ± 0.01 mg). Subsequently, all larvae were weighed and ration was adjusted to reflect the new weight. After 49 days of rearing, all survival larvae were collected, weighted, and counted from each aquarium. Individual total length and body wet weight were also recorded. At the end of the experiment, survival fish were collected, counted from each replicate. Thus 30 larvae were removed from each replicate to carcass composition determination. After collection, samples were stored at -20 °C until biochemical analysis.

The growth indices and nutrient utilization parameters were calculated for each treatment as follows: body weight gain (BWG) (g) = final body weight - initial body weight; specific growth rate (SGR) (%/day) = $\ln(\text{final body weight}) - \ln(\text{initial body weight}) \times 100/\text{number of day}$; feed conversion ratio (FCR) = total weight of feed consumed (g)/wet biomass gain (g); protein efficiency ratio (PER) = weight gain (g)/protein intake (g); survival rate (SR) (%) = $(\text{final number of larvae}/\text{initial number of larvae}) \times 100$; cannibalism rate (CR) (%) = $(\text{number of larvae missing}/\text{initial number of larvae}) \times 100$.

Water quality parameters

Water quality parameters were monitored during rearing period. Water temperature, dissolved oxygen, and pH were measured daily 8.00h using Oxy meter (WTW OXI 330) and pH meter (WTW pH 330 respectively. Phosphorus, total ammonia-N nitrate-N, and nitrite-N were measured once weekly using HACH DR/2000 spectrophotometer by the method of Golterman *et al.* (1978). The mean data of physicochemical parameters of water measured in the aquarium were showed in Table 3 and Table 4 respectively for Experiment 1 and Experiment 2.

Biochemical analysis

The approximate composition of experimental diets and the fish carcass were analyzed using standard methods (AOAC, 1995). Moisture content of each sample was determined through a hot-air oven set at 105 °C for 24 hours, and ash was determined by incineration at 550 °C in a muffle furnace for 24 hours. Crude proteins (nitrogen x 6.25) was determined using micro-Kjeldahl method; crude fat was extracted (hexane extraction) by using the Soxhlet method and crude fiber was quantified by acid digestion followed by ashing the dry residue at 550 °C in a muffle furnace for 4 hours.

Statistical analysis

Data on weight and length were transformed in $\log(x + 1)$ to have a normal distribution. The specific growth rate, body weight gain, coefficient of variance, Cannibalism rate, and Survival rate were analyzed using one-way analysis of variance (ANOVA). The Tukey's multiple range test and Duncan's multiple-range test were used to compare differences among treatment means. Treatment effects were considered significant at $p < 0.05$. The analyses were performed using Statistica 7.1 software.

III. RESULTS

The growth performances of *Heterobranchus longifilis* larvae fed *Artemia salina*, fish meal, beef brain, soybean, and blood sausage of pig and maggot flour recorded in Experiment 1 are shown in Table 5. Final body weight (FBW), final body length (FBL), specific growth rate (SGR), coefficient of variation (CV), cannibalism rate (CR) and survival rate (SR) showed significant differences among diets regimes. Highest BWG was found in larvae fed with ASA-, FME- and MFL-diets (2.1 ± 0.77 ; 2.03 ± 0.94 and 1.5 ± 0.4 g respectively) while the lowest BWG were recorded in larvae fed BSA- (0.28 ± 0.03 g) and SBE-diets (0.33 ± 0.03 g). Highest SR ($72.00 \pm 1.11\%$) and lowest CR ($8.00 \pm 1.25\%$) were obtained with larvae

fed MFL diet. Cannibalism rate ($49.33 \pm 1.4 \%$) was highest in fish fed FME diet. Feed conversion ratio (3.38 ± 0.39 ; 5.30 ± 1.610) values were significantly ($p < 0.05$) lowest in fish fed MFL and ASA respectively. Inversely, Protein efficiency ratio (1.47 ± 0.20) was highest in fish fed BBR.

In Feeding with supplemented diets, the growth performances of larvae fed *Artemia salina* (ASA), beef brain (BBR+), soybean (SBE+) and maggot flour (MFL+) were shown in Table 6. Significant differences ($p < 0.05$) were recorded in Final body length (FBL), Final body weight (FBW), Body weight gain (BWG) and Specific growth rate (SGR) among groups. FBL, FBW, BWG and SGR were highest in fish fed MFL+. Survival rate (SR) showed significant difference among groups. The highest value of SR was recorded in larvae fed MFL+ ($70.67 \pm 8.31 \%$) followed by ASA ($61.33 \pm 5.06 \%$), BBR+ ($52.00 \pm 5.31\%$) and SBE+ ($44.67 \pm 7.06 \%$). In addition, significant difference was recorded in Cannibalism rate (CR) among groups ($p < 0.05$). Larvae fed SBE+ showed the lowest value of CR ($14.00 \pm 3.05 \%$) followed by larvae fed MFL+ ($23.33 \pm 3.99 \%$). Feed conversion rate (0.89 ± 0.30) was lowest in fish fed MFL+. Protein efficiency ratio (0.95 ± 0.04) was lowest in fish fed SBE+.

Concerning carcass composition, the lipid content was significant higher ($p < 0.05$) in larvae fed SBE (Table 7). Proteins contents were highest in fish fed BSA-, ASA-, FME- and BBR-diets, among which no significant differences were found (Table 7). Carcass ash contents showed lower values in fish fed SBE ($2.67 \pm 0.20 \%$) and BSA ($3.14 \pm 0.16 \%$) diets. In addition, lower values of carcass dry matter were recorded with larvae fed BBR ($20.40 \pm 0.70 \%$) and SBE ($21.23 \pm 0.3 \%$) diets. In contrast, carcass dry matter values were highest in larvae fed BSA, FME, and MFL diets (Table 7).

In Feeding with supplemented diets, carcass composition showed significant difference ($p < 0.05$) among groups (Table 8). In larvae fed SBE+ carcass lipid ($9.97 \pm 1.81 \%$) and dry matter ($29.20 \pm 1.07 \%$) contents showed highest values. In contrast, carcass crude protein and ash contents were lowest in this group. No significant differences in protein contents were recorded among larvae fed ASA, MFL+ and BBR+, but in these groups, carcass dry matter showed highest values.

IV. DISCUSSION

Results of this study showed that diet influenced the growth performances and the survival of *Heterobranchus longifilis* larvae. The survival rate of larvae fed MFL was better than those recorded by ASA. The survival rates obtained with BBR ($54.67 \pm 1.63\%$) and MFL ($72.00 \pm 1.11\%$) as superior to 50% indicate that BBR and MFL diets can be use in *H. longifilis* long periods rearing. All groups of larvae grew during the 49 reared days, attesting the excellent growth potential feed utilization characteristics of this species (Kerdchuen and Legendre, 1992). Best utilization of diet (3.38 ± 0.39) was recorded in fish fed MFL. Initial feeding is a critical period during the early life of fish. Feeding *Artemia salina nauplii* to

larvae takes advantage of the larva's innate predatory nature to capture a mobile, live food particle (Knud-Hassen *et al.*, 1990). The suitability of *Artemia* as food for catfish larvae could also be due to its nutritive value (Wickins and Lee, 2002). Similar results were reported by Atsè *et al.* (2009) in *H. longifilis*. Verreth *et al.* (1993) reported that *Artemia salina nauplii* were generally accepted as an initial feed for good growth and survival of *Clarias gariepinus* and were recommended before weaning to dry food. The lower survival rate of larvae fed FME could be attributed to cannibalism exerted by the big size of larvae. It was shown that a significant heterogeneity of size of fish can lead to the cannibalism (Dabrowski *et al.*, 1985).

In the first Experiment, the BWG did not show any significant difference between *Artemia salina* and both FME and MFL diets. This similar growth shown between FME and MFL could constitute as well a good food for the rearing larvae as *Artemia salina*. The improved growth in fish fed MFL could be due to high quality of this diet, which may be related to its more palatability and digestibility (Sackey, 1989). *Heterobranchus longifilis* larvae fed on BBR had significantly improved growth than when fed on soybean and blood sausage of pig. The lower growth observed in larvae fed BSA diet despite of the high protein content could be to the anti-nutritional factors (Wilson and Poe, 1985). All diets used in this study had higher protein values to cover the *Heterobranchus longifilis* larvae needs which extends between 32-42.5% (Eyo, 1995). The carcass proximate protein of *Heterobranchus longifilis* larvae showed similar trends with diet protein contents. High diet protein content entrains high larvae carcass protein content in fish fed BSA. In the other hand, growth was lowest in this group. Maximizing the utilization of dietary protein for growth is related to both the dietary inclusion level of protein and the availability of non-protein energy sources, such as lipid and/or carbohydrate (Helland and Grisdale-Helland, 1998).

Concerning the supplemented diets, an improved larvae growth performance and carcass composition were observed in all groups. Moreover, larvae fed MFL+ had the highest final body weight (3.2 ± 0.80 g) compared to ASA, BBR+ and SBE+ diets. When MFL and BBR diets are supplemented, the cannibalism rate increased consequently the survival rate dropped, while the CV did not show any significant difference between groups. The supplementation of by-products improved diet utilization and protein efficiency ratio by two or three times in all diets in terms of average final body weight and weight gain. The carcass protein content was lower in fish fed SBE+ than other groups. On the other hand, the inclusion of minerals, vitamins and amino-acids in diets improves the carcass lipid content of larvae in all diets groups. This may be due to an increased lipogenesis in body tissues of fish. Increasing dietary crude ash contents at MFL+, BBR+ and SBE+ enhanced body ash content in larvae. Similar results were reported by Pouomogne *et al.* (1997) in tilapia.

This study showed that in the Experiment1, larvae fed with *Artemia salina* and FME had higher growth performances than other by-products. However, the supplementation of diets with minerals, premix vitamin and amino acids increases larvae growth performances and survival. Moreover, the supplementation of the maggot diet improves the growth and survival better than *Artemia salina* diet.

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Table1: Proximate composition of diets used in Experiment 1 (% dry matter).

| Component | ASA | FME | BSA | SBE | BBR | MFL |
|-----------------------------------|-------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Dry matter | 54.7±0.61 ^b | 96.73±0.20 ^c | 22.73±0.60 ^a | 92.41±0.20 ^c | 21.8±0.90 ^a | 91.99±0.70 ^c |
| Crude protein | 49.00±1.10 ^c | 61.29±0.90 ^d | 75.25±1.60 ^c | 37.63±0.50 ^a | 36.50±0.80 ^a | 42.00±1.40 ^b |
| Crude lipid | 1.98±0.05 ^a | 8.29±0.41 ^b | 2.74±0.04 ^a | 10.87± 0.30 ^c | 51.80±1.30 ^c | 28.95± 0.90 ^d |
| Ash | 2.78±0.10 ^a | 12.6±0.68 ^c | 4.82 ±0.88 ^b | 10.1±1.52 ^d | 1.96 ±0.02 ^a | 8.10± 1.10 ^c |
| Fiber | 5.13±0.80 | 0.02±0.01 | 0.01±0.00 | 6.50 ±1,49 | 0.01±0.01 | 5.89±0.42 |
| NFE | 41.11±1.50 ^d | 17.82± 0.99 ^b | 17.19±1.52 ^b | 34.9±2.81 ^c | 9.74±1.12 ^a | 15.06 ±2.82 ^b |
| Gross energy (kJg ⁻¹) | 18.72±1.47 ^a | 19.90±1.91 ^a | 20.73±1.63 ^{ab} | 18.58±1.77 ^a | 29.93± 2.03 ^c | 23.18±1.52 ^b |
| EPR (kcalg ⁻¹) | 7.13±1.03 ^b | 6.50±2.63 ^{ab} | 5.44±0.15 ^a | 9.61±1.28 ^c | 18.02±2.37 ^d | 11.68±1.85 ^c |

Artemia salina = ASA, Fish meal = FME, blood sausage of pig = BSA, soybean = SBE, beef brain = BBR, maggot flour = MFL. NFE (%) = Nitrogen free extract; EPR (kcalg⁻¹) = Energy-protein ratio (kcalg⁻¹)

Table 2: Proximate composition of diets used in Experiment 2 (% dry matter)

| Component | ASA | SBE+ | BBR+ | MFL+ |
|-----------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Dry matter | 54.7±0.61 ^b | 90.89±0.90 ^c | 21.38±1.50 ^a | 89.88±1.78 ^c |
| Crude protein | 49.00±1.10 ^c | 34.81±0.49 ^a | 32.95±0.67 ^a | 41.17±0.90 ^b |
| Crude lipid | 1.98±0.05 ^a | 10.41±0.90 ^b | 48.3±1.78 ^d | 24.88±0.35 ^c |
| Ash | 2.78±0.10 ^a | 10.89±0.30 ^b | 2.32±0.11 ^a | 10.53±0.60 ^b |
| Fiber | 5.13±0.80 | 5.65±0.99 | 0.01±0.01 | 5.01±0.71 |
| NFE | 41.11±1.50 | 38.24±1.68 | 16.43±1.56 | 18.41±1.44 |
| Gross energy (kJg ⁻¹) | 18.72±1.47 ^a | 18.35±1.17 ^a | 28.93±2.57 ^b | 21.98±2.07 ^a |
| EPR (kcalg ⁻¹) | 7.13±1.03 ^a | 10.16±3.79 ^a | 19.07±2.16 ^b | 11.16±2.31 ^a |

Artemia salina = ASA, soybean fortified = SBE+, beef brain fortified = BBR+, maggot flour fortified = MFL+, NFE (%) = Nitrogen free extract; EPR (kcalg⁻¹) = Energy-protein ratio (kcalg⁻¹)

Tableau 3: Physicochemical parameters of water tanks in experiment 1

| Paramters | ASA | FME | BSA | SBE | BBR | MFL |
|--|-------------|-------------|-------------|-------------|-------------|-------------|
| T (°C) | 30.1 ± 0.99 | 29.5 ± 1.08 | 30.2 ± 1.03 | 30.0 ± 1.14 | 29.7 ± 0.79 | 30.0 ± 0.63 |
| O ₂ (mgL ⁻¹) | 5.84 ± 0.36 | 5.56 ± 0.31 | 5.13 ± 0.38 | 5.47 ± 0.51 | 5.33 ± 0.45 | 5.47 ± 0.55 |
| pH | 7.71 ± 0.20 | 7.84 ± 0.22 | 7.63 ± 0.39 | 7.71 ± 0.20 | 7.73 ± 0.34 | 7.60 ± 0.19 |
| NO ₂ ⁻ N (mgL ⁻¹) | 0.61 ± 0.07 | 0.60 ± 0.11 | 0.65 ± 0.05 | 0.53 ± 0.13 | 0.58 ± 0.06 | 0.62 ± 0.09 |
| PO ₄ ³⁻ N (mgL ⁻¹) | 0.25 ± 0.05 | 0.28 ± 0.05 | 0.36 ± 0.33 | 0.27 ± 0.09 | 0.28 ± 0.07 | 0.31 ± 0.10 |
| NH ₄ ⁺ (mgL ⁻¹) | 0.31 ± 0.08 | 0.31 ± 0.05 | 0.50 ± 0.17 | 0.34 ± 0.12 | 0.40 ± 0.06 | 0.32 ± 0.11 |

Artemia salina = ASA, Fish meal = FME, blood sausage of pig = BSA, soybean = SBE, beef brain = BBR, maggot flour = MFL

Tableau 4: Physicochemical parameters of water tanks in experiment 2

| Paramters | ASA | SBE+ | BBR+ | MFL+ |
|--|------------|-----------|-----------|-----------|
| T (°C) | 30.20±1.09 | 30.7±1.43 | 30.4±1.11 | 30.5±1.17 |
| O ₂ (mgL ⁻¹) | 5.80±0.36 | 5.13±0.35 | 4.47±0.55 | 6.56±0.31 |
| pH | 7.60±0.20 | 7.41±0.29 | 7.31±0.20 | 7.83±0.32 |
| NO ₂ ⁻ N (mgL ⁻¹) | 0.58±0.01 | 0.65±0.07 | 0.49±0.13 | 0.66±0.11 |
| PO ₄ ³⁻ N (mgL ⁻¹) | 0.24±0.05 | 0.34±0.13 | 0.17±0.02 | 0.18±0.04 |
| NH ₄ ⁺ (mgL ⁻¹) | 0.31±0.03 | 0.51±0.09 | 0.34±0.12 | 0.34±0.08 |

Artemia salina = ASA, soybean fortified = SBE+, beef brain fortified = BBR+, maggot flour fortified = MFL+

Table 5: Growth parameters of *Heterobranchus longifilis* larvae fed for 49 days with *Artemia salina* and five by-product

| Paramters | ASA | FME | BSA | SBE | BBR | MFL |
|---------------------------|-------------------------|----------------------------|--------------------------|-------------------------|-------------------------|--------------------------|
| FBW (g) | 2.10±0.77 ^c | 2.03±0.94 ^c | 0.29±0.09 ^a | 0.34±0.06 ^a | 0.82±0.31 ^{ab} | 1.5±0.40 ^{bc} |
| FBL (mm) | 65.95±8.85 ^c | 64.50±21.0 ^c | 29.35±12.61 ^a | 27.83±5.25 ^a | 46.19±9.54 ^b | 46.10±3.40 ^b |
| BWG (g) | 2.09±0.26 ^c | 2.02±0.73 ^c | 0.28±0.03 ^a | 0.33±0.03 ^a | 0.81±0.23 ^b | 1.49±0.28 ^{bc} |
| SGR (%day ⁻¹) | 11.43±0.26 ^c | 11.38±0.66 ^c | 7.10±0.92 ^a | 7.80±0.13 ^a | 9.77±0.49 ^b | 10.80±0.42 ^{bc} |
| CV (%) | 38.09±0.73 ^c | 46.27±2.66 ^d | 27.01±0.79 ^b | 19.07±1.06 ^a | 37.67±0.83 ^c | 26.73±2.37 ^b |
| CR (%) | 27.33±1.11 ^d | 49.33±1.40 ^c | 25.33±0.70 ^d | 18.00±0.60 ^b | 21.33±1.36 ^c | 8.00±1.25 ^a |
| SR (%) | 64.00±2.47 ^c | 9.33±1.15 ^a | 28.00±0.62 ^b | 43.33±0.16 ^c | 54.67±1.63 ^d | 72.00±1.11 ^f |
| FCR | 5.30±1.61 ^{ab} | 10.48 ± 1.57 ^{cd} | 13.47±2.05 ^d | 7.89±2.89 ^{bc} | 8.52±1.97 ^{bc} | 3.38±0.39 ^a |
| PER | 0.70±0.04 ^c | 0.16±0.06 ^a | 0.36±0.06 ^b | 0.36±0.02 ^b | 1.47±0.20 ^d | 0.76±0.27 ^c |

Artemia salina = ASA, Fish meal = FME, blood sausage of pig = BSA, soybean = SBE, beef brain = BBR, maggot flour = MFL.

Final body weight = FBW, Final body length = FBL, Body weight gain = BWG, Specific growth rate = SGR, Coefficient of variation = CV, Cannibalism rate = CR, Survival rate = SR, Feed conversion ratio = FCR, Protein efficiency ratio = PER.

^{a,b,c,d,e,f} letters in the same row show differences among treatments ($p < 0.05$)

Table 6: Growth parameters of *Heterobranchus longifilis* larvae fed for 49 days with *Artemia salina* and three supplemented by-products

| Paramters | ASA | SBE+ | BBR+ | MFL+ |
|-----------|--------------------------|---------------------------|----------------------------|---------------------------|
| FBW (g) | 2.02 ± 0.54 ^b | 0.89 ± 0.23 ^a | 1.48 ± 0.43 ^b | 3.20 ± 0.80 ^c |
| FBL (mm) | 65.95±8.85 ^c | 49.25 ± 4.80 ^a | 55.91 ± 3.90 ^{ab} | 72.94 ± 6.33 ^c |
| BWG (g) | 2.01 ± 0.02 ^b | 0.87 ± 0.14 ^a | 1.47 ± 0.09 ^b | 3.19 ± 0.71 ^c |

| | | | | |
|---------------------------|----------------------------|---------------------------|---------------------------|---------------------------|
| SGR (%day ⁻¹) | 11.09 ± 0.60 ^{bc} | 9.54 ± 0.55 ^a | 10.44 ± 0.10 ^b | 11.98 ± 0.59 ^c |
| CV (%) | 26.73 ± 4.23 | 26.34 ± 2.62 | 29.70 ± 3.15 | 26.73 ± 2.37 |
| CR (%) | 30.66 ± 5.79 ^c | 14.00 ± 3.05 ^a | 32.67 ± 7.26 ^c | 23.33 ± 3.99 ^b |
| SR (%) | 61.33 ± 5.06 ^c | 44.67 ± 7.06 ^a | 52.00 ± 5.31 ^b | 70.67 ± 8.31 ^d |
| AFRCR | 3.27 ± 1.13 ^b | 3.31 ± 0.63 ^b | 3.28 ± 1.16 ^b | 0.89 ± 0.30 ^a |
| PER | 1.14 ± 0.03 ^b | 0.95 ± 0.04 ^a | 4.32 ± 1.76 ^c | 3.04 ± 0.86 ^c |

Artemia salina = ASA, soybean fortified = SBE+, beef brain fortified = BBR+, maggot flour fortified = MFL+. Final body weight = FBW, Final body length = FBL, Body weight gain = BWG, Specific growth rate = SGR, Coefficient of variation = CV, Cannibalism rate = CR, Survival rate = SR, Feed conversion ratio = FCR, Protein efficiency ratio = PER.

^{a,b,c,d} letters in the same row show differences among treatments ($p < 0.05$)

Table 7: Proximate composition of *Heterobranchus longifilis* larvae fed on *Artemia salina* and five by-products (% wet matter)

| Composition | ASA | FME | BSA | SBE | BBR | MFL |
|---------------|--------------------------|--------------------------|-------------------------|--------------------------|--------------------------|-------------------------|
| Dry matter | 22.6±0.30 ^b | 26.01±1.50 ^c | 31.1±1.60 ^d | 21.23±0.30 ^{ab} | 20.4±0.70 ^a | 25.83±0.40 ^c |
| Crude protein | 15.75±1.10 ^{cd} | 15.75±1.06 ^{cd} | 16.63±1.30 ^d | 11.38± 0.90 ^a | 14.98±1.20 ^{bc} | 13.13±1.00 ^b |
| Crude lipid | 1.24±0.07 ^b | 1.46±0.03 ^b | 1.18±0.01 ^{ab} | 6.24±1.90 ^c | 0.82±0.01 ^a | 1.89±0.02 ^b |
| Ash | 5.42±0.10 ^b | 5.31±0.10 ^b | 3.14±0.16 ^a | 2.67± 0.20 ^a | 5.59±0.35 ^b | 5.03±0.09 ^b |

Artemia salina = ASA, Fish meal = FME, blood sausage of pig = BSA, soybean = SBE, beef brain = BBR, maggot flour = MFL ^{a,b,c,d} letters in the same row show differences among treatments ($p < 0.05$)

Table 8: Proximate composition of *Heterobranchus longifilis* larvae fed on *Artemia salina*, beef brain fortified, soybean fortified, and maggot flour fortified (% wet matter)

| Composition | ASA | SBE+ | BBR+ | MFL+ |
|---------------|--------------------------|-------------------------|-------------------------|-------------------------|
| Dry matter | 21.20± 0.90 ^a | 29.2±1.07 ^c | 25.66±0.50 ^b | 24.87±1.00 ^b |
| Crude protein | 16.63±1.18 ^b | 13.13±0.88 ^a | 15.75±1.42 ^b | 15.75±1.21 ^b |
| Crude lipid | 5.11±1.34 ^b | 9.97±1.81 ^c | 4.17±1.29 ^a | 1.89±0.02 ^b |
| Ash | 5.64±0.90 ^b | 3.78±0.80 ^a | 5.76± 0.50 ^b | 5.87±0.17 ^b |

Artemia salina = ASA, soybean fortified = SBE+, beef brain fortified = BBR+, maggot flour fortified = MFL+.

^{a,b,c} letters in the same row show differences among treatments ($p < 0.05$).