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Effect of Maggot Dietary Protein Level on Growth Performance, Feed Utilization, Survival Rate and Body Composition of *Heterobranchus longifilis* Larvae Reared in Aquarium

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Authors' contributions

This work was carried out in collaboration between all authors. Author YBO designed the study, wrote the protocol and the first draft of the manuscript; authors BCA and ARK managed the analyses of the study; author LPK managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: This study is aimed to investigate the effects of maggot crude protein levels (25 %, 30%, 35% and 40%) on growth performance, feed utilization, survival rate and body composition Larvae of *Heterobranchus longifilis.*
Study Design: The experiment was carried out in aquaria (38.5 x 46.5 x 28 cm^3), stocking each aquarium with 50 larvae (1larvae/L). Four experimental diets were formulated based on the maggot meal as the main protein source. These diets were formulated at 25, 30, 35 and 40% protein levels with maggot meal and maize flour as the major ingredients.
Place and Duration of Study: This study was carried out in the reproduction laboratory, at Oceanological Research Center, Abidjan, Ivory Coast between the periods of March to May 2011.
Methodology: The diets were offered to the larvae (initial mean weight 0.004±0,001g) three times per day *ad libitum* for 49 days.
Results: The results showed that the growth indices such as final body weight, weight gain, and specific growth rate increased significantly with increasing maggot protein level to

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a maximum at 35% of maggot protein showed insignificant decrease in growth indices. Feed conversion ratio and protein efficiency ratio were better in 35% of maggot protein level. The highest values of survival rate were recorded with fish fed 35% and 40% maggot protein. Concerning body composition of larvae moisture and ash were not affected by dietary maggot protein level. The protein content of larvae increased with increasing of dietary protein level while the lipid content decreased.
Conclusion: From the present results, diet containing 35% of maggot protein is considered optimal for *Heterobranchus longifilis* larvae.

Keywords: Maggots; protein; Heterobranchus longifilis; larvae; growth; body composition.

1. INTRODUCTION

Feed accounts for a large percentage of total cost in aquaculture and protein is the most expensive component of aquaculture diets [1]. Commercial aquatic feeds have traditionally been based on fishmeal as the main protein source because it's high protein content and balanced essential amino acid profile. Fishmeal is also a good source of essential fatty acids, digestible energy, minerals and vitamins. However, fishmeal is relatively expensive; supply is limited and quality variable [2]. Consequently, the leveling out of annual fishmeal supplies, coupled with the increased demand for fishmeal in feeds for livestock and poultry, is likely to reduce the availability of fishmeal in aquatic feeds [3]. In addition, fishmeal is one of the most expensive ingredients in formulated feeds. So to reduce feed costs, aquaculturists need to replace fishmeal with alternative protein sources [4,1].

Fingerling production to supply the farms has become a major headache because of the high cost of fish dietary protein sources because larvae fish protein requirements are higher, and their feed formulation requires the use of high quality dietary protein [5]. Nauplii of *Artemia salina* are generally used as the main protein source for first-feeding in larvae fish nutrition due to its high protein content, palatability and highly digestibility to most fresh water and marine fish [6,7]. However, *Artemia salina* is not usually available particularly in the developing countries [6]. This makes intensive fish cultivation very expensive in these countries where aquaculture is not sufficiently developed. This outlook has therefore given impetus to the research for alternative protein sources. Recent studies have shown that maggot meal produced from the larval stage of the housefly *Musca domestica* posses a great potential [8,9]. It is rich in protein (39-55%), phosphorus, trace elements and B complex vitamins [9,10]. Maggot meal has been evaluated as replacement of fishmeal in feed for livestock [11, 10]. Maggot meal is used as protein source in fish diet for tilapia [8,9] and catfish [12,13]. It was observed that maggot meal can replace some levels of fishmeal dietary inclusion depending on fish species and size. For Heteroclarias, (*Heterobranchus longifilis* x *Clarias gariepinus*) fingerlings, 25 % maggot meal inclusion had the best growth performance [12]. Concerning *Clarias gariepinus* juveniles (100-120 mg), 50% maggot meal in diet recorded the highest weight gain [13]. Also the introduction of maggot meal in the diet of African catfish larvae provides a good opportunity to develop low-cost food especially for tropical countries where *Artemia salina* is not available locally. Otherwise, because protein is the essential ingredient of fish feeds, it is important to accurately determine the protein requirements for fish species and sizes in aquaculture. This study is to determine the optimal dietary maggot protein level for growth of *H. longifilis* larvae.

2. MATERIALS AND METHODS

2.1 Ingredients Characterization

The maggot meal used in this study was processed from maggots cultured from poultry droppings and fish carcass [14]. All feed-grades ingredients including maize flour, palm oil, lysine, methionine, vitamin, amino acid premix and compounded mineral such as iron, chloride and phosphorus were purchased from local markets in Abidjan (Côte d'Ivoire). The proximate composition of maggot meal and maize flour are given in Table 1.

Table 1. Composition of the ingredients used in the experimental diets

Ingredients (%)	Maggot meal	Maize flour
Moisture	08.01	09.08
Crude protein	42.00	09.50
Lipid	28.95	05.22
Total carbohydrate	15.06	81.01
Ash	08.10	01.63
Fibers	05.89	02.64

2.2 Experimental Diets

Four experimental diets were formulated based on maggot meal as the main protein source (Table 2). These diets were formulated at 25, 30, 35 and 40% protein levels with maggot meal and maize flour as the major ingredients. These two raw materials were combined in different proportions. Lysine and methionine were added at a level of 2.00 and 2.13% respectively to meet the nutritional requirements of the *Heterobranchus longifilis* larvae. Phosphorous, iron and chloride were added at 0.67% level. Palm oil, vitamin and amino acid premix were included at 2% level. The feed ingredients were ground using a homogenous mixture grinder. Diets were processed by blending the dry ingredients for 15 min. Water ($800mL.kg^{-1}$) at 80ºC was added to the mixture during the blending process and mixed for 15 min. The paste obtained was dried in electric oven at 60ºC for 48 hours, crushed into fine powder and passed through a fine mesh screen. Then, the experimental diets at 250-300 µm size were stored at -20ºC until use.

2.3 Experimental Fish and Feeding Trial

The experiment was carried out in the reproduction laboratory, at Center of Oceanological Research, Abidjan, Ivory Coast. African *Heterobranchus longifilis* larvae used in this study were obtained by using the procedure of reproduction already established [15]. Three days after hatching, a total of 600 *H. longifilis* larvae with an average weight of 4.00 ± 1.00 mg were equally distributed over 12 glass aquaria (38.5 x 46.5 x 28 cm^3) stocking 50 larvae per aquarium (1 larva L^{-1}). All aquaria were filled with filtered freshwater and aerated. Before starting the trial, fish were acclimated to experimental condition for 4 days. During the acclimation period, larvae were fed *Artemia salina ad libitum*. After this period, the four treatments were assigned randomly in triplicates to the aquaria and larvae were fed *ad libitum* (100% total biomass). Three times a day (08:00, 12:00 and 17:00 h), rations of the experimental diets were weighed and distributed several times throughout the day during 49 days. Every day, the dead larvae were removed from aquaria and counted. Three times a week, undigested food particles and waste products were siphoned out before feeding fish,

and uneaten food particles were regularly dried and weight for feed conversion ratio calculation. During the feeding period, every week, the total weight and number of fish were measured in each aquarium to adjust the feed ration. At the end of the experiment, all fish were collected, counted and individually weighed. Then, 30 larvae were removed from each replication to chemical composition determination.

Water temperature, dissolved oxygen, pH, phosphorus, total ammonia-N nitrate-N, and nitrite-N were monitored during rearing period. Water temperature was recorded daily using a mercury thermometer suspended in each aquarium. Dissolved oxygen, and pH were recorded daily at 7.30 h 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 [16].

Table 2. Formulation and proximate composition of the experimental test diets

	Dietary protein level (%)			
Ingredients (%)	**25**	**30**	**35**	**40**
Maggot flour	50.55	66.00	81.15	89.00
Maize flour	39.71	24.26	8.76	1.26
Crude palm oil	2.00	2.00	2.00	2.00
Lysine	2.13	2.13	2.13	2.13
Methionine	1.61	1.61	1.61	1.61
VMD-Aminovit (Premix)[1]	2.00	2.00	2.00	2.00
Phosphorus	0.67	0.67	0.67	0.67
Iron	0.67	0.67	0.67	0.67
Chlorine	0.66	0.66	0.66	0.66
Total	100	100	100	100
Proximate analysis				
Moisture	11.74	10.70	10.66	10.33
Crude protein (% DM)	25.18	30.89	35.07	40.08
Total fat (% DM)	18.86	22.68	26.06	25.94
Ash (% DM)	5.32	6.16	7.25	8.15
Crude fiber (% DM)	4.34	4.93	5.53	5.82
Nitrogen free extract (NFE) (% DM)[2]	34.56	24.66	15.43	9.68
Gross energy (kJg^{-1})[3]	18.87	19.92	20.57	20.65
P/E ($g.kJ^{-1}$)[4]	1.33	1.55	1.70	1.94

[1]Composition for 1 kg of premix : Vitamin A = 10000 UI, Methionine = 50.0 mg, Vitamin D3 = 1000 UI, Vitamin E = 10.0 mg, Vitamin B1 = 2.0 mg, Vitamine B2 = 4.0 mg, pantothenic Calcium = 10.0 mg, Vitamin B6 = 1.5 mg, Vitamin C = 25.0 mg, Vitamin K3 = 1.5 mg, Acide folique = 0.5 mg, Nicotinamide = 20.0 mg, Biotine = 15.0 µg, Lysin HCl = 50.0 mg, Alanin = 12.96 mg, Arginin = 15.6 mg, Aspartic Acid = 27.8 mg, Cystine = 1.9 mg, Glutamic Acid = 85.0 mg, Glycin = 8.0 mg, Histidin = 11.8 mg, Isoleucin = 23.6 mg, Leucin = 35.4 mg, Phenylalanin = 19.0 mg, Prolin = 392 mg, Serin = 24.0 mg, Threonin = 18.6 mg, Tryptophane = 6.4 mg, Valin = 27.4 mg; [2]Nitrogen-free extract (NFE) = 100 - (% protein + % lipid + % moisture + % ash + % fiber); [3]Gross energy = % protein x 22.2 kJ/g + % lipid x 38.9 kJ/g + % Nitrogen-free extract x 17.2 kJ/g; [4]P/E = Protein to energy ratio in g protein / kJ gross energy.

2.4 Growth Indices and Nutrient Utilization

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; daily weight gain (DWG) (g/day) = (final body weight - initial body weight)/(number of day); specific

growth rate (SGR) (%/day) = [ln (final body weight) – ln (initial body weight)] x 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; protein efficiency ratio (PER) = weight gain (g)/protein intake (g); survival rate (SR) (%) = (final number of larvae/initial number of larvae) x 100; cannibalism rate (CR) (%) = (number of larvae missing/initial number of larvae) x 100; mortality rate (MR) (%) = (number of dead larvae/initial number of larvae) x 100.

2.5 Biochemical Analysis

The approximate composition of experimental diets and the fish carcasses sample were determined according to the Association of Official Analytical Chemists[17]. Moisture was determined after oven drying (105ºC) for 24 h (MEMMERT Drying Oven, GE-174, Memmert GmbH, Heilbronn, Germany). Ash was measured by incineration at 550ºC in a muffle furnace for 24 h (Thermo Fisher Scientific Heraeus M 110 Muffle Furnace, Waltham, MA, USA). Crude protein was determined using micro-Kjeldahl method, N% x 6.25 (Kjeltech autoanalyzer, Model 1030, Tecator, Höganäs, Sweden), crude fat by soxhlet extraction with hexane (Soxtec System HT6, Tecator), crude fibre by acid digestion followed by ashing the dry residue at 550ºC in muffle furnace for 4 h. The gross energy contents of the diet and fish were calculated on the basis of their crude protein, total fat and carbohydrate contents using the equivalents of 22.2, 38.9 and 17.15 kj g^{-1}, respectively [18].

2.6 Statistical Analysis

Results were statistically analyzed with a one-way analysis of variance (ANOVA) using Statistica version 7.1 software package. Duncan's multiple range test was used to compare difference between treatments means when significant F-values were observed. All percentage and ratio data were arc-sin transformed before analysis [19]. The treatment effects were considered to be significant at $p < 0.05$. Non-transformed data are presented in Tables to simplify comparisons.

3. RESULTS

3.1 Water Quality

Water quality parameters during the 49-days experimental period were observed to be normal for all aquaria. Water temperature was maintained at 28.44 ± 0.02ºC, Dissolved Oxygen 5.94 ± 0.14 mg L^{-1}, pH 7.21 ± 0.14, ion ammonium-N ranged from 0.33 to 0.45 mg L^{-1}, nitrite-N at 0.63 ± 0.04 mg L^{-1} and phosphorus at 0.18 ± 0.02 mg L^{-1}. Data remained within ranges allowing for high growth rate and production for most aquaculture species [20].

3.2 Growth Performance

The growth performance, feed utilization efficiency and survival rate for *H. longifilis* larvae fed different maggot dietary protein levels are shown in Table 3. Final body weight (FBW), body weight gain (WG), daily weight gain (DWG) and specific growth rate (SGR) were significantly ($p< 0.05$) affected by the levels of maggot dietary protein. The lowest significant ($p< 0.05$) values of these growth indices was observed for *H. longifilis* larvae fed the diet containing 25% protein level, while the highest values was recorded for larvae fed diet containing 35% maggot protein following by diets containing 40% and 30% maggot protein

levels, though growth indices values obtained for larvae fed 30%, 35% and 40% maggot crude protein were not significantly different. SGR increased with increasing dietary maggot protein levels to a maximum at 35% maggot protein levels and decreased slightly at 40% dietary maggot protein (Fig. 1). Maximum values of FBW, WG and SGR were recorded for larvae fed diet containing 35% dietary protein (Table 3).

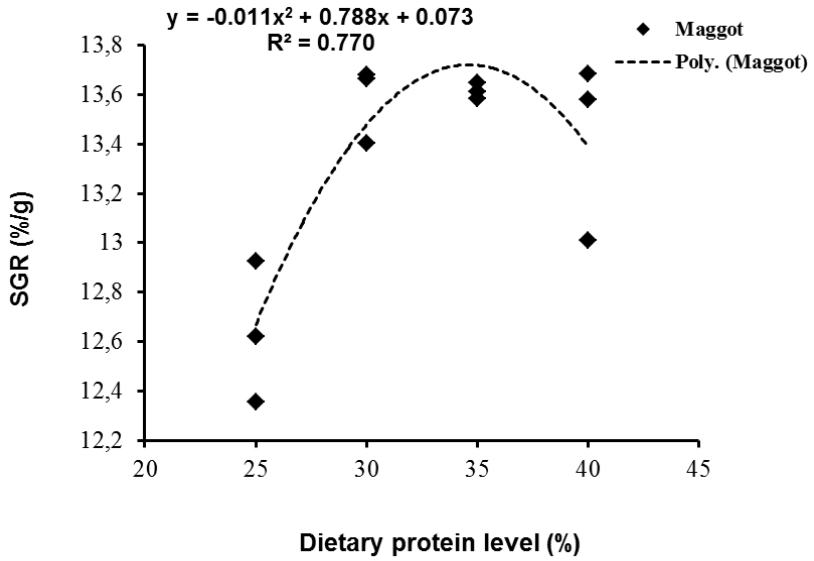

Fig. 1. Effects of dietary protein level on weight gain of *H. longifilis larvae* fed experimental diet.

3.3 Nutrient Utilization Indices

Feed conversion ratio (FCR) and protein efficiency ratio (PER) were significantly ($p< 0.05$) affected by the levels of maggot dietary protein (Table 3). FCR in the diets containing 35% (1.02 ± 0.00) and 40% (1.04 ± 0.01) protein levels were significantly lower ($p< 0.05$) than in the diets containing 25% (1.18 ± 0.03) and 30% (1.10 ± 0.01) protein levels. The highest significant value ($p< 0.05$) of PER (7.03 ± 0.71) was observed for *H. longifilis* larvae fed the diet containing 35% protein level, while the lowest values were recorded for larvae fed diets containing 25% (5.5 ± 0.19), 30% (5.47 ± 0.54), and 40% (5.92 ± 0.89) protein levels.

3.4 Survival, Cannibalism and Mortality

Average survival rate (SR), cannibalism rate (CR) and mortality rate (MR) of *H. longifilis* larvae fed with the different maggot dietary protein levels are shown in Table 3. Survival rate ranged from 54.00% to 70.67%. The highest significant ($p<0.05$) values of SR was observed for larvae fed the diets containing 35% (70.67 ± 1.61 %) and 40% (69.33 ± 1.62%) protein levels, followed by larvae fed the diet containing 30% (60.00 ± 2.3%) protein level. The lowest significant ($p<0.05$) SR value (54.00 ± 1.12) was reported from larvae fed the diet containing 25% protein level. Larvae fed diets containing 25% and 30% protein levels recorded the significant ($p<0.05$) highest values of CR contrary to those of larvae fed with 35% and 40% dietary protein levels. MR was significantly ($p<0.05$) higher in larvae fed the diet containing 25% protein level than larvae fed 30%, 35% and 40% dietary protein levels.

Table 3. Growth performance, nutrient utilization and survival rate of larvae *H. longifilis* fed the experimental diets

Parameters	Dietary protein level (%)			
	25	30	35	40
IBW (g)	0.004±0.001[a]	0.003±0.001[a]	0.004±0.001[a]	0.004±0.001[a]
FBW (g)	1.91±0.11[a]	2.73±0.08[b]	3.01±0.12[b]	2.99±0.27[b]
WG (g)	1.90±0.28[a]	2.73±0.17[b]	3.00±0.31[b]	2.99±0.20[b]
DWG (g day^{-1})	0.038±0.004[a]	0.06±0.01[b]	0.06±0.01[b]	0.06±0.01[b]
SGR (% day^{-1})	12.63±0.29[a]	13.58±0.16[b]	13.62±0.03[b]	13.42±0.86[b]
FCR	1.18±0.03[c]	1.10±0.01[b]	1.02±0.00[a]	1.04±0.01[a]
PER	5.50±0.19[a]	5.47±0.54[a]	7.03±0.71[b]	5.92±0.89[a]
CR (%)	27.33±1.06[b]	28.00±2.01[b]	20.00±1.40[a]	18.98±1.57[a]
MR (%)	18.67±2.81[b]	12.00±3.46[a]	9.33±3.71[a]	11.69±1.85[a]
SR (%)	54.00±1.12[a]	60.00±2.30[b]	70.67±1.61[c]	69.33±1.62[c]

IBW = initial body weight, FBW = final body weight, WG = weight gain (g), DWG = daily weight gain (g day^{-1}), SGR = specific growth rate (% day^{-1}), FCR = feed conversion ratio, PER = protein efficiency ratio, CR = cannibalism rate, MR = mortality rate, SR = survival rate

Mean values ± SD in the same row sharing the same superscript are note significantly different (P < 0.05).

3.5 Proximate Composition of Larvae

Whole-body composition data are presented in Table 4. No significant differences among treatments were detected for body moisture and ash contents (%). In contrast fish protein, lipid and energy contents were significantly ($p< 0.05$) affected by the levels of maggot dietary protein. The lowest ($p< 0.05$) protein content was recorded for the diet containing 25% protein level, while the highest value was recorded for larvae fed diet containing 40% maggot protein level, though values obtained for larvae fed 30% and 35% maggot crude protein were not significantly different of protein content value obtained in larvae fed diet containing 40% maggot protein. The opposite trend was observed for whole body energy. Whole lipid content significantly ($p<0.05$) decreased with increasing of dietary protein levels. The highest significant ($p< 0.05$) value of lipid content (6.34 ± 0.25 %) was observed for *H. longifilis* larvae fed with diet containing 25% protein level, while the lowest value (4.43 ± 0.07%) was recorded for larvae fed diet containing 40 % protein level.

Table 4. Whole body composition of *H. longifilis* larvae fed different experimental diets (wet-weight basis)

Composition	Dietary protein level (%)			
	25	30	35	40
Moisture (%)	75.42±0.63[a]	75.85±0.77[a]	76.02±0.81[a]	76.09±0.28[a]
Ash (%)	4.62±0.10[a]	4.73±0.03[a]	4.74±0.11[a]	4.69±0.18[a]
Crude protein (%)	13.62±0.14[a]	14.25±0.08[b]	14.48±0.12[b]	14.81±0.31[b]
Crude lipid (%)	6.34±0.25[c]	5.17±0.21[b]	4.76±0.14[b]	4.43±0.07[a]
Gross energy (KJ g^{-1})	5.49±0.19[b]	5.17±0.10[a]	5.07±0.08[a]	5.14±0.10[a]

Mean values ± SD in the same row sharing the same superscript are note significantly different (P < 0.05).

4. DISCUSSION

At the end of the trial, the feed conversion ratio values (1.02, 1.04, 1.10 and 1.18) recorded in this study were lower than 1.33-2.25 reported for *Heterobranchus longifilis*[21] and 1.5 – 2 [5] as the best feed conversion ratio for the good growth of most species in aquaculture. However, the best survival rate was obtained in larvae fed 35% maggot dietary protein. In add the growth rate, weight gain, specific growth rate and nutriment utilization increased progressively with dietary protein level to a maximum at 35%. This observation was in agreement with that observed in *Heterobranchus longifilis* [21] and in *Heterobranchus bidorsalis* [22]. These authors observed increasing growth with the increase in dietary protein level in these two species. In fact, because protein is the most essential component in the carnivorous fish diets, dietary protein must be in accordance to the protein requirements for larvae of *H.longifilis* to improve growth. Similarly, a diet with inadequate protein content can result in reduced weight gain because the fish cannot eat enough feed to satisfy their nutriment requirements for growth. In this study, considering polynomial regression analysis of SGR, growth results and nutrient utilization indicate that the dietary protein requirement of larvae *H. longifilis* is 35%. Our results are different from those of some authors who reported respectively 40% and 42.5% protein levels for good growth of *H. longifilis* larvae [21,23]. The optimum dietary protein levels observed in fish species depend on the source of protein, fish size and age, and feed quality [5]. 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. Inclusion of non-protein energy has been shown to spare dietary protein from catabolism to provide energy and enhance its utilization for growth, a process known as protein-sparing [24]. In this study, the good growth performances observed from larvae of *H. longifilis* fed with diet containing 35% protein level could be due to the good nutritional quality of the nutriment contained in the experimental diets and the good biological value of dietary protein provided to larvae by maggot meal. In fact, housefly maggot meal has a balanced and rich amino acid profile and it is rich in methionine [9]. Maggot meal is also rich in phosphorous, trace elements and B complex vitamins [10]. Based on cost effectiveness, availability and crude protein content, maggot meal seems to be a good protein source for *H. longifilis* larvae. However, maggot protein was more efficiently utilized by larvae of *H. longifilis* to a maximum at 35% crude protein level. Above 35% protein, growth decreased slightly, showing the low utilization of dietary protein. This result demonstrated that 35% protein level is the optimum for *H. longifilis* larvae growth. Beyond this limit, the excess protein could lead to reduced growth performance [4]. The decrease in growth could be due to energy requirement for metabolism, rather than for protein deposition [4]. Otherwise, it was observed that the high dietary protein contents (35% and 40%) decreased significantly the rate of cannibalism and increased the survival rate of larvae. In add, final body weight (FBW = 3.01 ± 0.12 g); cannibalism rate (CR = 20,00 ± 1.40%), and survival rate (SR = 70,67 ± 1,67 %) obtained in the larvae fed the diet containing 35% maggot dietary protein are greater than those obtained (FBW = 1.10 ± 0.03 g; CR = 20.49 ± 6.53%; SR = 69.20 ± 6.70) with the reference food *Artemia salina* in the same culture conditions [6]. These results demonstrate the best quality and palatability of the diet formulated based on the maggot meal at 35% protein level for *Heterobranchus longifilis* larvae. This diet could replace *Artemia salina* in *H. longifilis* larvae feeding for improve growth.

Biochemical analysis of *H. longifils* larvae showed that the increase in dietary protein level in diets formulated maggot meal based did not influence moisture and ash contents. In contrast, fish protein content increased concomitantly with the decrease of body lipid when the dietary protein increases. This relationship was also noted in juvenile monosex Nile

tilapia [25]. The increase of whole body protein and decrease of lipid content with increasing dietary protein level may be attributed to the high carbohydrate and low protein content of the diet containing 25% of protein level. The excess carbohydrate in the diet may be converted into body fat for storage [25]. In addition, the biochemical composition showed that the highest lipid content was recorded for the larvae fed diets containing low protein levels (25% and 30%). This observation agrees with other studies which reported that diets with high lipid content alter body composition of fish, by reducing their water content [26].

5. CONCLUSION

In conclusion, the present study shows that an intensive rearing up to 49 days with good survival and growth rates can be carried out using diet containing 35% maggot crude protein for African catfish *Heterobranchus longifilis* larvae.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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