

Original Research Article

Growth response and nutrient utilization of *Clarias gariepinus* juvenile fed *Bombyx mori* (Mulberry silkworm) diet as protein source.

ABSTRACT

A 56-day (Eight weeks) trial was carried out to determine the growth and nutrient utilization of *Clarias gariepinus* juveniles fed *Bombyx mori* meal (BMM) as a replacement for fishmeal. Five diets were formulated which included 100% fishmeal, 25% BMM inclusion, 50% BMM inclusion, 75% BMM inclusion and 100% BMM inclusion designated diets A, B, C, D and E respectively with the fish fed at 5% body weight. The effect of the diets on the growth, carcass and mineral compositions of the fish were evaluated. The results showed that mean weight gain, specific growth rate and protein efficiency ratio of fish fed the experimental diets differed significantly ($P > 0.05$) when compared with the control. In this study, replacement of 25% fishmeal gave the best result while 100%, 75% and 50% gave better result than the control. Also, carcass yield and mineral depositions differed marginally in the fishes fed the various diets. It can be inferred from this study that *B. mori* larvae have the potential to be used as a protein source and can replace and elicit better growth trends in fish more than the conventional fish meal without any problem with growth.

Keywords: Growth response, Nutrient utilization, *Bombyx mori*, *Clarias gariepinus*

INTRODUCTION

The rapid world-wide expansion of aquaculture to meet up with the global demand of fish is a strong indicator that crisis will be precipitated in the aqua feed industries in the near future. Since many fisheries are overfished or heading towards depletion, aquaculture is recognized as the only feasible option to meet the increasing demands for aquatic foods (Allan, 2004; Chebbaki *et al.*, 2010). Feed is one of the major inputs in aquaculture production, and fish feed technology is one of the least development sector of aquaculture particularly in Africa and other developing countries of the world (Gabriel *et al.*, 2007). It was reported by Ahmad and Diab (2008) that the feeding cost in aquaculture represents over 50% of the operation cost of fish farming. Fishmeal

as a raw material is the choice in aquaculture due to its high quality protein with balanced amino acid profile (Gatlin *et al.*, 2007) and according to Goytortu'a, (2006), the production of fishmeal has been relatively stable in the last two decades but the increasing demand could not be matched in the present world due to increased aquaculture. Moreover, the cost of fishmeal is increasing day by day which renders the use of alternative protein source which are local, cheap and unsuitable for direct human consumption inevitable to make up for the shortage of fishmeal and secure the stable supply for commercial feeds (Hardy, 2005)

Many works have been done on the nutritional value of other source of raw materials to supplement the dietary fishmeal-based diets. Research has shown that alternative protein sources derived from grains such as corn, wheat, barley, soybean, cotton seed, fishmeal, and animal rendering products such as mechanically deboned meal, poultry by-product meal, meat and bone meal, maggot, Bambara groundnut, etc. can be used to formulate nutritious aquaculture feed at various levels of inclusions in different feeds. Among animal and plant protein sources, Yang (2006) reported that diets with 20% of fishmeal replaced by meat- and- bone meals produced growth equivalent to the control diet in *Gibel carp*. Kasper *et al.* (2007) concluded that up to 47.6% of the fishmeal could be replaced with soy bean meal in the diets of yellow perch (*Perca flavescens*) without affecting the feed consumption, weight gain, feed efficiency or survival of the fish. Toledo *et al.* (1986 & 1987) partially replaced 15% of fishmeal with shrimp head meal in *Oreochromis aureus* feed without any adverse effect on feed and growth efficiency. *Nibeamiichthioides* and *Carassius auratus gibelio* grew well on diets with 50% of the fishmeal replaced by poultry- by- product meal (Wang, 2006; Yang, 2004). Also, Oso *et al.* (2013) in a study showed that *Clarias gariepinus* fingerlings fed up to 75% inclusion of Bambara groundnut in fish feed was satisfactorily acceptable without affecting growth and feed utilization.

Insects are important source of food to many animal species including man (Adedire and Aiyesanmi, 1999). Their larvae may constitute a cheaper source of essential nutrients that are easily available and affordable to the natives within the localities where the insect larvae are found and could form a base for new food products of considerable nutritive value (Ekpo and Onigbinde, 2007). *Bombyx mori* can be used for the production of silk and are also proteinaceous in nature. These worms originated from China with four developmental stages and undergo complete metamorphosis (Krishnawami, 2005). The larvae undergo spinning, vomiting the silk

fibre into liquid form around themselves to form cocoon so that they will not emerge out and develop into adult stage called moth. The dead larvae in the cocoon are removed by riling machine and the silk is separated while the dead larvae are used for production of fish feed. The objective of this study is to determine the optimum level of fishmeal replacement with *Bombyx mori* as protein source in the diet of *Clarias gariepinus*. The aim is to evaluate the response in terms of growth of *Clarias gariepinus* juvenile fed *Bombyx mori* as a protein source at different levels of inclusion so as to make cheaper and qualitative supplementary feeds available to culture fish species with a view to bridging the gap between protein supply and consumption through reduction in cost of production in the aquaculture sector.

MATERIALS AND METHODS

Feed Preparation:

The feed ingredients used were fishmeal, *Bombyx mori*, maize, salt, soya bean, groundnut cake, vitamin, premix, lysine and methionine. The feed ingredients were purchased from Metro Vet Consultant Limited in Ekiti State while the *Bombyx mori* used were purchased from Ekiti State Sericulture Project. The larvae were killed during the process of cooking so that they will not emerge out of the cocoon and to avoid damage to the silk. Inside the cocoon, the larvae were oven dried at 65⁰C and were later removed from the cocoon, pulverized and analyzed for proximate composition according to the procedure of APHA (2006) as shown in table 1. Feed ingredients were finely ground and mixed together in appropriate proportions after which the mixture was pelleted into small size and then sun dried.

Experimental Site- The experiment was carried out at the postgraduate research laboratory of Zoology Department, Ekiti State University, Ado-Ekiti for a period of 56 days (eight weeks). Sixteen plastic bowls of 20 litres water holding capacity were used for the experiment. Water was maintained at 17 litres level throughout the experiment and a mosquito mesh was used to cover the bowls to preclude predators from entering into the bowls and fish jumping out as well.

Experimental Fish- *Clarias gariepinus* juvenile were used for the experiment. One hundred and fifty mixed sex juvenile with average weight of 7.20g were obtained from a hatchery in Ado-

Ekiti. The fish were acclimatized for two days in a plastic bowl during which they were not fed in order to empty their gut and prepare their appetite for the new feed. The fish were randomly stocked at the rate of ten (10) juveniles per bowl. The initial mean weight of fish in each bowl was taken and recorded. Fish were fed at 5% of their body weight twice on daily basis, that is, morning (08:00 – 09:00 am) and evening (17:00 – 18:00 pm). Subsequent weight and standard length measurements were taken every week and the rations fed were adjusted accordingly to fish weight gain. General observations were made daily for mortality and also movement and the behavior of fish were monitored. Water in the experiment bowls was renewed totally on daily basis to remove unconsumed food particles and faecal materials to prevent poor water quality.

Experimental Design and Diets – The experiment consisted of five treatments with three (3) replicates representing each treatment including control. Each treatment diet contained *Bombyx mori* as fishmeal substitute except the control. The four diets were formulated to contain varying percentage of *Bombyx mori* fortified with different ingredients as shown in Table 2. Each of the diets was analyzed to determine the proximate composition. The various diets were designated as follows at varying levels of *Bombyx mori* inclusion: 0%, 25%, 50%, 75% and 100% for Diets A (control), B, C, D and E respectively.

Sampling- A weekly sampling was carried out to determine the new growth rate and survival using manual weighing balance.

Physico-chemical Parameter of Water- The following parameters were monitored during the experiment: temperature, pH, and dissolved oxygen.

Feed Evaluation Parameters- The initial and weekly mean weights were recorded per treatment.

Weight gain (WG) = Final weight of fish – Initial weight of fish

Percentage weight gain (%WG) =
$$\frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

125

$$\text{Percentage specific growth rate (\%SGR)} = \frac{(\text{Log } W_2 - \text{Log } W_1) \times 100}{T_2 - T_1}$$

127

128 Where:

129 W_2 = Weight of fish at time T_2 (final)130 W_1 = Weight of fish at time T_1 (initial)131 T = Period of experiment in days

132

$$\text{Feed conversion ratio (FRC)} = \frac{\text{Dry weight of feed}}{\text{Total weight gained by fish}}$$

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$$\text{Protein efficiency ratio (PER)} = \frac{\text{Total weight gained by fish}}{\text{Protein intake}}$$

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139 **Statistical Analysis**

140 The growth performance and feed utilization parameters were analyzed by univariate analysis
 141 using SPSS version 17. Univariate analysis is able to analyze two parameters simultaneously to
 142 see if there are strong and meaningful links between them. The significant difference used was at
 143 the 0.05 level.

144

145 **RESULTS**

146 Table 3 shows mean growth rate, feed conversion ratio and protein efficiency ratio of *Clarias*
 147 *gariepinus* juveniles fed the five different diets (A, B, C, D and E) for eight weeks. The result
 148 shows the weight gain, % weight gain, the specific growth rate (SGR), protein efficiency ratio
 149 and feed conversion ratio. The highest weight gain was recorded in fish fed with diet B (3.05)
 150 followed by diet E (2.85) while the least value was recorded in diet C (2.33). Fish fed with diet B
 151 had the highest increase in percentage body weight gain (45.05) followed by diet E (38.41). The
 152 lowest value of 31.14 was recorded in diet A. A similar trend was observed for the specific
 153 growth rate and protein efficiency ratio in which the highest mean of 2.00 and 0.086 was
 154 recorded with diet B followed by diet E (1.76 and 0.080). Fish fed with diet A gave the least
 155 S.G.R. and protein efficiency ratio (1.47 and 0.064) respectively. The highest value of 4.35 was

achieved with the fish fed diet D followed by diet C (3.84) in feed conversion ratio. The lowest value (2.79) was recorded with diet E.

Proximate composition of experimental feed is shown in table 4. The ash content was very high in diet A and low in diet D with 11.95 and 9.84 respectively while the percentage fat was high in diet E (3.70) and low in diet A (3.563). The highest and least crude protein was recorded in diet A (37.70) while 35.46 was recorded for both diet D and E. Diet C and B gave the highest (7.37) 6.74) and least (6.40) 42) for moisture content and percentage fibre respectively. The percentage soluble carbohydrate was high in diet B (37.73) and low in diet A (35.21).

The proximate composition of *Clarias gariepinus* juvenile flesh is shown in table 5. The highest ash content was recorded in diet D (16.44) and the least in diet E (15.49) while the highest moisture content was recorded in diet A (11.41) and least in diet D (10.26). Diet E gave the highest crude protein (67.54) while the least was recorded in diet D (66.34). The highest and least percentage fat was recorded in diet E and C with 5.76 and 5.39 respectively. The percentage soluble carbohydrate was very high in diet C (1.80) and low in diet D (0.42). The experimental fish showed little weight gain. Low mortality was also observed in all the treatments throughout the period of the experiment.

Table 1: Proximate Composition of *Bombyx mori* larva

Components	% in larva
% dry matter	87.50
% crude protein	69.52
% crude fibre	5.01
% ether extracts	11.12
% ash	7.88

Table 2: Gross Composition of Experimental Diets (Kg)

INGREDIENTS	DIET A Control 0%	DIET B 25% <i>B- mori</i>	DIET C 50% <i>B- mori</i>	DIET D 75% <i>B- mori</i>	DIET E 100% <i>B- mori</i>
Fish meal 72 <i>B. mori</i>	10 –	7.5 2.5	5 5	2.5 7.5	– 10
Maize	11	11	11	11	11
Rice bran	7	7	7	7	7
Fish meal 65	16	16	16	16	16
Soya bean	15	15	15	15	15
Groundnut	38	38	38	38	38
Bone meal	1	1	1	1	1
Salt	1.33	1.33	1.33	1.33	1.33
Lysine	0.37	0.37	0.37	0.37	0.37
Methionine	0.1	0.1	0.1	0.1	0.1
Vitamin C	0.1	0.1	0.1	0.1	0.1
Vit premix	0.1	0.1	0.1	0.1	0.1
Total	100	100	100	100	100

Table 3: Mean Growth Rate and Feed Conversion Ratio of *Clarias gariepinus* Juvenile.

Parameters	Diet A	Diet B	Diet C	Diet D	Diet E
Initial weight	7.29 ^a	6.77 ^b	7.17 ^a	7.33 ^a	7.42 ^a
Final weight	9.56 ^b	9.82 ^{ab}	9.50 ^b	9.77 ^{ab}	10.27 ^a
Weight gain	2.27 ^{de}	3.05 ^a	2.33 ^d	2.44 ^c	2.85 ^b
% Weight gain	31.14 ^d	45.05 ^a	32.50 ^{cd}	33.29 ^c	38.41 ^b
Specific growth rate	1.47 ^d	2.00 ^a	1.53 ^{bc}	1.56 ^{bc}	1.76 ^b
Protein efficiency Ratio	0.064 ^c	0.086 ^a	0.065 ^c	0.068 ^c	0.080 ^{ab}
Feed conversion ratio	3.55 ^c	3.12 ^d	3.84 ^b	4.35 ^a	2.79 ^e

Means with the same alphabet across columns are not significantly different at P< 0.05

Table 4: Proximate Composition of Experimental Feed

Parameters	Diet A	Diet B	Diet C	Diet D	Diet E
Ash	11.95 ^a	10.24 ^b	11.35 ^{ab}	9.84 ^c	10.23 ^b
Moisture	6.54 ^d	6.40 ^e	7.37 ^a	6.86 ^c	7.22 ^b
Crude protein	35.70 ^a	35.60 ^b	35.50 ^c	35.46 ^d	35.46 ^d
Fat	3.53 ^c	3.63 ^b	3.58 ^b	3.60 ^b	3.70 ^a
Fibre	6.54 ^b	6.42 ^c	6.74 ^a	6.64 ^{ab}	6.57 ^b
CHO	35.21 ^d	37.73 ^a	35.76 ^c	36.25 ^b	37.72 ^a

Table 5: Proximate Composition of Experimental Fish

Parameters	Diet A	Diet B	Diet C	Diet D	Diet E
Ash	15.35 ^c	16.39 ^a	15.35 ^c	16.44 ^a	15.49 ^b
Moisture	11.41 ^a	11.38 ^a	10.64 ^{ab}	10.26 ^b	10.69 ^{ab}
Crude protein	66.49 ^c	66.79 ^b	66.62 ^b	66.34 ^d	67.54 ^a
Fat	5.59 ^b	5.42 ^c	5.39 ^c	5.57 ^b	5.76 ^a
CHO	0.86 ^b	0.41 ^d	1.80 ^a	0.42 ^d	0.53 ^c

DISCUSSION AND CONCLUSION

In the present study, fish fed the various diets showed increase in weight without any external sign of nutritional deficiency because growth performances of fish fed the *Bombyx mori* diets with up to 100% replacement were similar and even better in terms of weight gain, % weight gain, specific growth rate and protein efficiency ratio when compared with the control. This shows the possibility of using *B. mori* as alternative protein source in the diet of *C. gariepinus* as it contains all the necessary nutrients that are required for growth. Orilogbon *et al* (2011) reported that insect meals have a higher level of methionine, lysine and valine compared to soybean meal. The study showed significant differences ($P < 0.05$) in the growth performance of

Clarias gariepinus fed the experimental diet at various inclusion of *B. mori* as diet B, E, D and C were shown to be more significant in decreasing order than diet A (control) in terms of weight gain, percentage weight gain, specific growth rate and protein efficiency ratio. This result contradicts the work done by Oyegoke (2006) on dietary potentials of the edible larvae of *Cirina forda* (westwood) as poultry feed; he stated that there were no significant differences between the growth performances of the broiler chicks as better results were obtained from fish fed with the experimental diet. The best performance of fish was gotten from 25% inclusion of BMM. This supports the work of Agbese (2006) that replacement of fishmeal with 25% maggot meal gave the best performance in the fish. Also, more than 25% BMM inclusion gave a better growth performance when compared with control. This contradicts the report made by Agbese (2006) that higher replacement levels than 25% magmeal gave a lower growth.

The *Clarias gariepinus* juvenile fed diet D (i.e. 75% inclusion of *Bombyx mori*) showed superior feed conversion ratio. This agreed with the findings of Davies & Gouveia (2010) who worked on the dietary nitrogen utilization in African cat fish fed oil seed meal diets under sub-optimal growth condition consistent with a restricted regime. It was stated that fish fed the experimental diets showed superior feed conversion ratio. The result of the proximate composition of carcass of fish fed experimental diets showed significant difference at various levels of *B. mori* inclusion. This however disagreed with the work done by Nwanna (2003) where he reported no significant difference ($P < 0.05$) in the carcass composition and mineral deposition of *Clarias gariepinus* fed chemically preserved shrimp head waste silage diets. Also, the higher lipid deposits in the carcass of fish fed experimental diets showed better growth in terms of weight which may be due to lipid rather than crude protein gain as this supports the report of Adeparusi and Agbede (2004) that better performance in growth, nutrient utilization and carcass crude protein deposit in *O. niloticus* fed diets with leaf protein cake showed that leaf proteins made from *Gliricidia* and *Leucaena* could be viable means of improving fish feed especially for herbivorous fish like *O. niloticus*.

Also, the result of this study showed the preference of *Clarias gariepinus* juvenile for small amount of lipids in their diet for growth because the highest weight was observed in fish fed diet B. This may be attributed to the fact that the lipids of animal origin such as silkworm have

shorter chain and lower degree of fatty acids rather than Omega 6-fatty acid in contrast to terrestrial animal which require long chain of omega 6- fatty acids (Tanazawa, 1993).

It can be concluded from the present study that 25%, 100%, 75% and 50% of *Bombyx mori* respectively could replace fish meal protein in the diet of *Clarias gariepinus* juvenile as it was satisfactorily acceptable to the fish without affecting the growth and feed utilization. This will ultimately reduce the cost of fish meal to be included in the diet and consequently reduce the cost of fish production. It can be said that whatever was responsible for a drop in weight gain and feed utilization of fish fed diet C (50% *B. mori* inclusion) could be taken care of by using a better processing method. Moreover, *B. mori* replacement can be tested on other fish species, poultry and any other animal diets.

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