Original Research Article

Growth performance and nutrient utilization efficiency of *Clarias gariepinus* juvenile fed *Bombyx mori* (Mulberry silkworm) meal as a partial replacement for fishmeal.

ABSTRACT

A 56-day (Eight weeks) trial was carried out to determine the growth performance and nutrient utilization of *Clarias gariepinus* juveniles fed *Bombyx mori* meal (BMM) as a replacement for fishmeal. Five experimental diets were formulated at various levels of inclusion of 25%, 50%, 75% and 100% of BMM designated as diets B, C, D and E respectively. The control diet (diet A) was 100% fishmeal. The experimental fish were collected from a private hatchery in Ado Ekiti and stocked in 20-litre plastic bowls at a stocking density of 10 fingerlings per bowl and fed twice per day at 5% body weight. The effect of the diets on the growth, feed utilization efficiency, 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 fishmeal with 25% BMM 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 fish 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 fishmeal 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 an indication of an impending pressure on the aqua-feed industries in the near future. Since many fisheries are overfished, 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 but the fish feed technology is one of the least developed 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). 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 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, 2006).

Many works have been done on the nutritional value of many alternative feed ingredients to supplement the dietary fishmeal-based diets. Several researches have shown that alternative protein sources derived from grains such as corn, wheat, barley, soy bean, 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. For instance, Yang (2004) 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) also reported that up to 47.6% of the fishmeal could be replaced with soybean meal in the diets of yellow perch (*Perca flavescens*) without affecting the feed consumption, weight gain, feed efficiency and survival of the fish. *Nibea miichthioides* and *Carassius auratus gibelio* grew well on diets with 50% of the fishmeal replaced by poultry- by- product meal (Wang *et al.* 2006; Yang *et al.*, 2004). Also, Oso *et al.*, (2013) 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.

Moreover, insects are important source of food to many animal species including man (Adedire and Aiyesanmi, 1999). Insect larvae, where they are available may constitute a cheaper source of essential nutrients that are easily available and affordable to the natives and could form a base for new food products of considerable nutritive value (Ekpo and Onigbinde, 2007). The larvae undergo spinning, vomiting the silk fibre into liquid substance 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 therefore 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 growth performance 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

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) from June 1 to July 26, 2012. Fifteen 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.

Feed Preparation:

The feed ingredients used for this study were purchased from Metro Vet Consultant Limited in Ekiti State while the *Bombyx mori* was purchased from Ekiti State Sericulture Project. The larvae of *Bombyx mori* were killed during the process of cooking so that they could be retained in the cocoon and thus, the silk was not damaged. Inside the cocoon, the larvae were oven dried at 65^0C and then removed from the cocoon. Later it was pulverized and analyzed for proximate composition using the procedure applied by APHA (2006) as shown in Table 1. Feed ingredients were finely ground and mixed together in appropriate proportions after which the mixture was pelleted (1.8mm diameter.) and then sun dried.

Table 1: Proximate Composition of *Bombyx mori* larvae

Composition	% in larvae
% dry matter	87.50
% crude protein	69.52
% crude fibre	5.01
% ether extracts	11.12
% ash	7.88

Experimental Fish- *Clarias gariepinus* juvenile were used for the experiment. One hundred and fifty mixed sex juveniles 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 a day, that is, morning from 08:00 – 09:00 am and evening from 17:00 – 18:00 pm. Subsequent weight and standard length measurements were taken every week and the rations of the feed were adjusted accordingly to fish weight gain. Mortality of the fish was recorded daily. In addition, water in the experimental bowls was renewed totally on daily basis to remove unconsumed food particles and faecal materials and thus good water quality parameters were maintained.

Experimental Design and Diets – The experiment consisted of five treatments with three (3) replicates. Each of the five experimental diets contained *Bombyx mori* as fishmeal substitute except the control. All the experimental diets were formulated to contain common basal diets and different proportions of *Bombyx mor*i as shown in Table 2. Each of the diets was analyzed to determine the proximate composition. The various diets were formulated to contain 0%, 25%, 50%, 75% and 100% *Bombyx mori* designated as Diets A (control), B, C, D and E respectively.

Table 2: Gross composition of experimental diets (Kg)

INGREDIENTS	DIET A Control 0%	DIET B 25% *B. mori*	DIET C 50% *B.mori*	DIET D 75% *B. mori*	DIET E 100% *B. mori*
Fish meal	10	7.5	5	2.5	–
B. mori	–	2.5	5	7.5	10
Maize	11	11	11	11	11
Rice bran	7	7	7	7	7
Blood meal	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
Vitamin premix	0.1	0.1	0.1	0.1	0.1
Total	100	100	100	100	100

Data collection and analysis

Sampling- All the fish were sampled every week to measure their body weight using manual weighing balance. Body length was measured using a measuring board to the nearest 0.1cm. Mortality of the fish was also recorded daily. In addition, physico-chemical parameters including temperature, pH and dissolved oxygen were monitored weekly during the experiment. Based on the data collected, growth performance, feed utilization efficiency and survival rate of fish were calculated as:

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

Percentage weight gain (%WG) = [(Final weight – Initial weight)/ Initial weight)] x 100

Percentage specific growth rate (%SGR) = $[(\text{Log } W_2 - \text{Log } W_1)/ T_2 - T_1)]x\ 100$

Feed conversion ratio (FCR) = Dry weight of feed/ Total weight gained by fish

Protein efficiency ratio (PER) = Total weight gained by fish/ Protein intake

Where: W_2 = Weight of fish at time T_2 (final)

W_1 = Weight of fish at time T_1 (initial)

T = Period of experiment in days

Statistical Analysis

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

RESULTS

The mean values of growth rate, feed conversion ratio and protein efficiency ratio of *Clarias gariepinus* juveniles fed the five experimental diets (A, B, C, D and E) for eight weeks were calculated and summarized in Table 3. The result shows that weight gain, % weight gain, the

specific growth rate (SGR), protein efficiency ratio and food conversion ratio were significantly affected by the type of feed provided for the fish. The highest weight gain was recorded in fish fed with diet B (3.05) followed by diet E (2.85) while the lowest value was recorded in diet C (2.33). Fish fed with diet B had the highest increase in percentage body weight gain (45.05) followed by diet E (38.41). The lowest value of 31.14 was recorded in diet A. A similar trend was observed for the specific growth rate and protein efficiency ratio in which the highest mean of 2.00 and 0.086 was recorded with diet B followed by diet E (1.76 and 0.080). Fish fed with diet A revealed the least S.G.R. and protein efficiency ratio (1.47 and 0.064) respectively. The highest value of feed conversion ratio (4.35) was achieved with the fish fed diet D followed by diet C (3.84) . Diet E gave the superior FCR of 2.79.

Table 3: Mean growth performance and feed utilization efficiency 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}
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}

Mean values with the same alphabets across rows are not significantly different at P> 0.05

The proximate composition of all the experimental feed is summarized 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 lowest crude protein was recorded in diet A (37.70) while 35.46 was recorded for both diets D and E. Diets C and B gave the highest (7.37 and 6.74) and lowest (6.40 and 6.42) moisture content and percentage fibre respectively. The percentage soluble carbohydrate was high in diet B (37.73) and low in diet A (35.21).

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]

Mean values with the same alphabets across rows are not significantly different at P> 0.05

The proximate composition of *Clarias gariepinus* juvenile flesh is summarized in Table 5. The highest ash content was recorded in diet D (16.44) and the lowest in diet E (15.49), while the highest moisture content was recorded in diet A (11.41) and lowest in diet D (10.26). Diet E had the highest crude protein (67.54), while the lowest was recorded in diet D (66.34). The highest and lowest percentage fat was recorded in diet E (5.76) and C (5.39) respectively. The percentage soluble carbohydrate was very high in diet C (1.80) and low in diet D (0.42). Low mortality was also observed in all the treatments throughout the period of the experiment. Diet D had the highest mortality of 13.33%, while Diet B recorded the lowest rate of 6.67%.

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]

Mean values with the same alphabets across rows are not significantly different at P> 0.05

DISCUSSION

In the present study, fish fed the various diets showed increase in weight without any external sign of nutritional deficiency even though growth performances of fish fed the *Bombyx mori* diets with up to 100% replacement varied 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 and Adewole (2011) reported that insect meals have a higher level of methionine, lysine and valine compared to soybean meal. The present study showed significant differences ($P<0.05$) in 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 than diet A (control) in terms of weight gain, percentage weight gain, specific growth rate and protein efficiency ratio. This result agrees with the work of Olaniyi and Babasanmi (2013) on silkworm meal used as a replacement for fishmeal in the diet of African Catfish up to 100%. The best performance of fish was observed in 25% inclusion of BMM. This supports the work of Sogbesan *et.al.* (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 Sogbesan *et.al.* (2006) that higher replacement levels than 25% maggot meal 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 and 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. This is supported by the report of Adeparusi and Agbede (2004) on growth, nutrient utilization and carcass crude protein deposit in

Oreochromis niloticus fed diets with leaf protein cake which showed that leaf proteins made from *Gliricidia* and *Leucaena* could be viable means of improving fish feed especially for herbivorous fish like *O. niloticus.*

The result of this study also showed the preference of *Clarias gariepinus* juvenile for small amount of lipids in their diet for growth because the highest growth performance 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 (Kanazawa and Fujimoto 1993).

CONCLUSION

In conclusion, although 25% gave the best result in this present study, however 100%, 75% and 50% levels of replacement of fish meal protein *Bombyx mori* in the diet of *Clarias gariepinus* juvenile are 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 later stage of the fish to make a general conclusion.

REFERENCES

Adedire C. O. and Aiyesanmi A. F. (1999). Proximate and mineral composition of the adult and immature forms of the variegated grasshopper, *Zonocerus variegatus* (L) (*Acridoidea: Pygomorphidae*). *Bioscience Research Communications.* 11(2):121-126.

Adeparusi E. O. and Agbede J. O. (2004). Evaluation of *leucaena* and *gliricidia* leaf protein concentrate as supplements to Bambara groundnut (*Vigna subterranea L. Verdc*) in the diets of *Oreochromis niloticus in 6th International Symposium of Tilapia in Aquaculture (ISTA).* 335-346.

Ahmad H. M. and Diab S. A. (2008). Replacement of fish meal protein by okara meal in practical diets for all male monosex Nile tilapia (*Oreochromis niloticus*). *8th International Symposium on Tilapia Aquaculture*. 729-737.

Allan J. D. (2004). Land Scapes and Riverscapes: The influence of Land Use on Streams and Ecosystems. *Annual Review of Ecology, Evolution, and Systematics.* 35: 257- 284.

American Public Health Association (APHA, 2006). Standard methods for the examination of water and waste water. *APHA Washington DC. 14th edition*. 107.

Chebbaki K., Akharbach H., Talbaoui E., Abrehouch A., Ait ali A., Sedki S., Ben Bani A., and Idaomar M.(2010). Effect of fish meal replacement by protein sources on the extruded and pressed diet of European sea bass juvenile (*Dicentrarchus labrax*). *Agriculture and Biology Journal of North America*. 1(4): 704-71.

Davies S. J. and Gouveia A. (2010). Response of common carp fry diets containing a pea seed meal (*Pisum sativum*) subjected to different thermal processing methods. *Elsevier*. 305(1-4): 117- 123.

Ekpo K. E. and Onigbinde A. O. (2007). Characterization of Lipids in Winged Reproductives of the Termite *Macrotermis bellicosus*. *Pakistan Journal of Nutrition*. 6 (3): 247-251.

Gabriel U. U., Akinrotimi O. A., Bekibele D. O., Onunkwo D. N. and Anyanwu P. E. (2007). Locally produced fish feed: potentials for aquaculture development in sub-Saharan Africa. *African Journal of Agricultural Research*. 2(7): 287-295.

Gatlin D. M. III., Barrows F.T., Brown P., Dabrowski k., Gaylord T. G., Hardy R.W., Herman E., Hu G., Krogdahl A., Nelson R., Overturf K., Rust M., Sealey W., Skonberg D., Souza E. J., Stone D., Wilson R., and Wurtele E. (2007). Expanding the utilization of sustainable plant products in aqua feeds: a review. *Aquaculture Research*. 38 (6): 551-579.

Goytortu'a B.E, Civera C. R., Rocha M. S. and Green Y. A. (2006). Partial replacement of red crab (*Pleuroncodes planipes*) meal for fish meal in practical diets for the white shrimp, *Litopenaeus vannamei*. Effects on growth and in vivo digestibility. *Aquaculture*. 256: 414-422.

Hardy R.W. (2006). Worldwide fish meal production outlook and the use of alternative protein meals for aquaculture. En: Editores: Sau'rez L. E. C., Marie D. R., Salazar M. T., Lo'pez M. G. N., Cavazos D. A. V., Ortega PCAGy. Avances en Nuticio' n Acui' cola VIII. *VIII Symposium International de Nuticio' n Acui' cola. Universidad Auto'noma de Nuevo Leo'n, Me'xico*. 410-419.

Kanazawa, A. and Fujimoto, K. (1993). Effects of endogenous and exogenous n-3 and n-6 fatty acids on microsomal synthesis of docosahexaenoc acid in vitro. *J Nutr Sci Vitaminol* 39(3): 253-62.

Kasper C. S., Watkins B. A. and Brown P. B. (2007). Evaluation of two soybean meals fed to yellow perch (*Perca flavescens*). *Aquaculture Nutrition*. 13(6): 431- 438.

Nwanna L. C. (2003). Nutritional Value and Digestibility of Fermented Shrimp Head Waste Meal by African Catfish Clarias gariepinus. *Pakistan Journal of Nutrition* 2(6): 339-345

Olaniyi, C.O. and Babasanmi, G.O. (2013) Performance characteristics of African catfish (*Clarias gariepinus*) fed varying inclusion levels of silkworm pupae (*Anaphe infracta*). *Bang.J.Anim.Sci* 42 (1):76-80

Orilogbon J. O. and Adewole A. M. (2011). Ethnoichthyological knowledge and perception in traditional medicine in Ondo and Lagos States, southwest Nigeria. *Egyptian Journal of Biology*. 13: (57-64).

Oso J. A., Edward J. B., Ogunleye O. A. and Majolagbe F.A. (2013). Growth Response and Feed Utilization of *Clarias gariepinus* Fingerlings Fed with Bambara Groundnut as protein source. *Journal of Natural Sciences Research*. 3(5): 84- 90.

Sogbesan, A. O.; N. Ajuonu, B. O. Musa and A. M. Adewole. 2006. Harvesting techniques and evaluation of maggot meal as animal dietary protein source for "*Heteroclarias*" in outdoor concrete tanks. *World Journal of Agricultural Sciences* 2 (4): 394-402.

Yang Y., Xie S., Cui Y., Lei W., Zhu X., Yang Y. and Yu Y. (2004). Effect of replacement of dietary fish meal by meat and bone meal and poultry by- product meal on growth and feed utilization of gibel carp, *Carassiusa uratus gibelio*. *Aquaculture Nutrition*. 10: 289- 294.

Wang Y., Guo J. L., Cui Z. H. and Bureau D. P. (2006). Replacement of fish meal by rendered animal protein ingredients in feeds for cuneate drum (*Nibea miichthioides*). *Aquaculture*. 252: 476- 483.