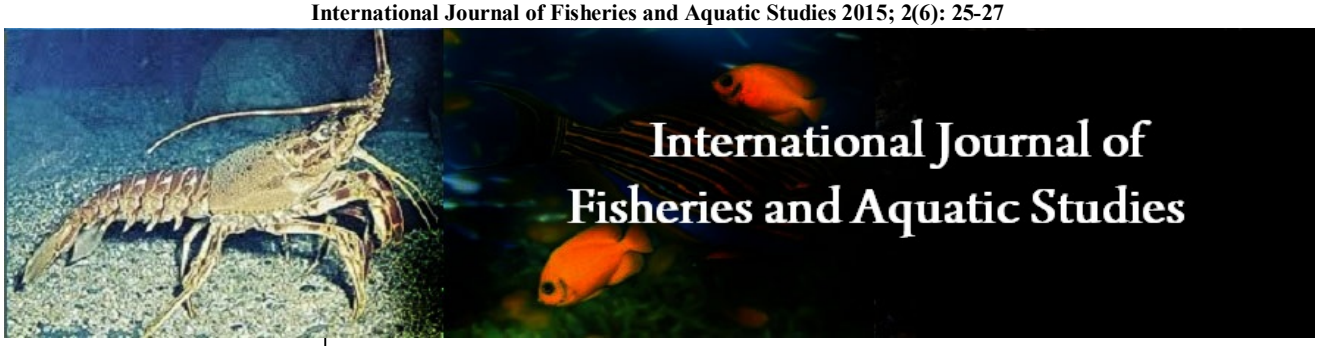

ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2015; 2(6): 25-27
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www.fisheriesjournal.com
Received: 06-05-2015
Accepted: 05-06-2015

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Effect of replacement of fish meal with silkworm (*Bombyx mori)* pupa protein on the growth of *Clarias gariepinus* fingerling

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Abstract
An experiment was conducted to determine the effect of replacement of fishmeal with silkworm (*Bombyx mori)* pupa protein (SPP) on growth and feed utilization of *Clarias gariepinus* fingerlings. Five isonitrogenous diets of about 40% crude protein with varying levels of replacement of fish meal (0-100%) were provided at 5 % live fish body weight for 40 days. Fish growth rate and feed utilization parameters was higher in fingerlings fed the diets with mixed fishmeal and SPP (the highest was 50:50) and lower in those fed 100 % of SPP or fishmeal. Significant differences were reported for weight gain, relative growth rate, food conversion ratio and protein efficiency ratio ($p > 0.05$) however, differences between SGR of experimental diets were insignificant ($p > 0.05$).

Keywords: Fish feeds, silkworm pupa protein, replacement of fishmeal, *Clarias gariepinus*

1. Introduction
In aquaculture, determinative share in running cost is expended on feeding the cultured fish. Protein is the most expensive component in fish feeds. Fish meal is most important feeding ingredient as dietary protein source for cultured fish. Shortage and high cost of fish meal make fish feed expensive hence the search for alternative sources of protein in fish feeds. Generally, the feed stuffs of animal origins are considered better alternative protein source if fish diets because of their higher content and other superior indispensable amino acids than the plant origins [1]. Several animal protein sources were evaluated to formulate the diets for fish such as poultry by-product meal, meat and bone meal, snail and other invertebrate meal [2, 3, 4, 5, 6]. Main tendency is to partial replacement of fish meal with alternative protein source [7, 8]. Insects may constitute a significant biomass. In Uzbekistan, silkworm (*Bombyx mori)* pupa has potency to use in fish feeding. Bombyx pupa is high valuable product used for production of chitosan [9]. In this production, there is such by-product as silkworm pupae protein (SPP). The SPP may be interesting for fingerling feeding. The present investigation aimed to evaluate the effect of fish meal replacement with silkworm (*Bombyx mori)* pupa protein in African catfish (*Clarias gariepinus*) fingerling diets on growth performance and nutritional value.

2. Materials and methods
An experiment was conducted from 23 July to 1 September 2014. Silkworm pupae protein were obtained as follows: mechanical cleaning of leftovers of pupae and adulterants; oil separation; deproteinization in solution NaOH of protein components; sulphate ammonium coagulation and separation of the protein by centrifugation; bleaching of protein; dialysis in flowing water, drying and preservation [10]. Amino acid analysis [11] was performed on an amino acid analyzer E 339 (Amino acid analyzer E 339, Mikrotechna - Prague-Czechoslovakia). In the protein 16 amino acids were identified, nine of which are irreplaceable - threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine, lysine, arginine. SPP comprises two protein components with molecular weights of 21.2 and 137.2 kDa. In quantitative ratio protein component with a molecular weight of 21.2 kDa was prevailed, which percentage was 89%. The high protein content with low molecular weight (about 90%) is perspective for fish feeding, as proteins of low molecular weight easily assimilated by the body. Toxicological analysis of protein from silkworm pupae was included determination of

the lethal dose by Litchfield-Wilcoxon method. Protein from silkworm pupae by the acute toxicity is belonged to the category of low-toxic substances [12].

One hundred and fifty same age and uniform size of African catfish fingerlings were obtained from scientific experiment station for aquaculture development located at Yangiyul district Tashkent region, Uzbekistan. The fingerlings were acclimatized into laboratory condition for one week before starting experiment and divided into five groups. Five diets of about 40 % crude protein were formulated. SPP was used to replace fishmeal at various inclusion levels of 0% (control), 25, 50, 75 and 100 %. The diets were coded as FM100, FM75, FM50, FM25 and FM0, respectively. The percentage of ingredients in each diet is shown in Table 1. Ten fingerlings of measured weight were carried in each glass tank. Each treatment had three replicates, and the fish from each replicate were held in a 50 x 50 x 50 cm tank. The fish were fed daily at 5% of their body weight at 8:00 12:00 16:00 and 20:00 for a period of 40 days.

Table 1: Percentage of ingredients in diets (%)

Ingredients	Diets				
	FM100 (control)	FM75	FM50	FM25	FM0
Fishmeal	58	43.5	29	14.5	0
SPP	0	14.5	29	43.5	58
Soybean meal (cake)	18.4	18.4	18.4	18.4	18.4
Sunflower cake	5.5	5.5	5.5	5.5	5.5
Wheat bran	10.1	10.1	10.1	10.1	10.1
Sunflower oil	6	6	6	6	6
Vitamin premix	2	2	2	2	2

The dry ingredients were ground to powder, the appropriate quantities for each diet were mixed with adding 10 % of water and pelleted in grinder with mesh diameter 1 mm. Pellets were dried, labeled and used for fish feeding.

Water temperature was kept as 26±1.5°C using aquaria thermostats in each tank. Dirt, uneaten feed, droppings and other particles were siphoned out from tanks daily. Each day 25 % of water was changed, while complete changing of water was done each 5 day.

Fish were weighed to the nearest to 0.1 g every week and daily rations were adjusted accordingly. Growth performance and feed utilization parameters were determined as follows:

- Weight gain (g) = $W_2 - W_1$, where W_1 (g) – the initial average (for each tank) body weight, W_2 (g) – the average (for each tank) final fish weight;
- Relative growth rate (g/day) = weight gain / t, where t – period in days;
- Specific growth rate (SGR) (% per day) = $[(\ln W_2 - \ln W_1) / t] * 100$, where ln – natural log;
- Feed conversion ratio (FCR) = feed intake (g) / wet body weight gain (g);
- Protein efficiency ratio (PER) = wet body weight gain (g) / protein intake (g).

Data were analyzed by one-way analysis of variance (ANOVA) with "R" statistical software. Statistical significance was determined at 5% (P<0.05).

3. Results

No fish died in all tanks, so survival rate was 100 %. Growth of average body weight during experiment is shown in Fig. 1. Average of initial weights, final weights, weight gains and other growth performance parameters of african catfish fed diets are shown in Table 2, so as growth performance and feed utilization parameters. As presented in Table 2, average weight gains (g) and relative growth rates (g/day) were significantly (p<0.05) higher in groups FM50, then FM75, and insignificantly higher at FM25, FM0 in compare with control (FM100) group. Specific growth rate (%/day) showed another results: fish at groups FM50 and FM0 had significantly higher results and in groups FM25, FM75 insignificantly higher in compare with control (FM100) group.

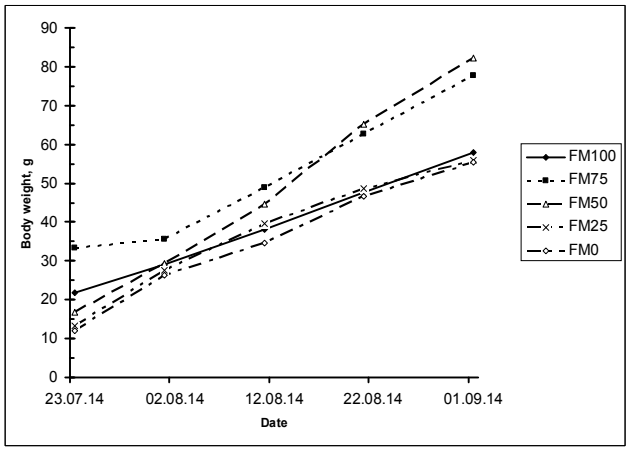

Fig 1: Growth pattern of *Clarias gariepinus* on different experimental diets

Results of feed quality showed following. All feed with replaced fishmeal had better quality for fish growth in compare with control feed; food conversion rates were significantly lower in groups FM50 and FM0 and insignificantly in groups FM75 and FM25 in compare with control (FM100). Protein efficiency rate was significantly higher in groups FM50 and FM0 and insignificantly higher in groups FM75 and FM25 in compare with control (FM100).

Analysis of variance showed significant influence of such factor as difference in diet (level of fishmeal replacement) to such parameters as weight gain, relative growth rate, food conversion ratio and protein efficiency ratio which were significantly different (p > 0.05) however, differences between SGR of experimental diets were insignificant (p > 0.05).

Table 2: Growth response and feed efficiency of *Clarias gariepinus* fingerling in fed experimental diets

Parameter	Diet				
	FM100	FM75	FM50	FM25	FM0
Initial weight (g)	2.2	2.3	1.7	1.3	2.3
Final weight (g)	5.8	7.8	9.0	6.5	6.8
Weight gain (g)	3.6	5.4[a]	6.7[a]	4.2	4.3
Relative growth rate (g/day)	0.91	1.36[a]	1.64[a]	1.07	1.08
SGR (% per day)	2.5	3.0	4.0[a]	3.5	3.8[a]
FCR	1.8	1.6	1.2[a]	1.5	1.4[a]
PER	1.4	1.6	2.1[a]	1.6	1.8[a]
[a] – mean significantly differ from the control at t-criteria (p>0.05)					

4. Discussion

This investigation indicated that progressive weight gain was reported in all treatments throughout the duration of experiment. That was the indication that the catfish responded positively to all the diets. Our initial hypothesis was that feed with replaced fishmeal will not have significantly lower fish growth parameters than control feed. But in experiment, fish

growth parameters in all groups with replaced fishmeal showed better result than in control group (FM100).

Growth rate was higher in group fed the diets with mixed fishmeal and SPP (the highest was FM50) and lower in those fed 100 % of SPP or fishmeal (FM0 and FM100, respectively). The results proved that partial replacement of fishmeal with SPP produced higher growth performance to those fed with single fishmeal.

Faturati *et al*. [13] and Akiwanda *et al*. [14] reported that feed with protein content 39-41% has preferable level of quality for feeding of African catfish. Diets used in our experiments have 39-40 % of protein content.

In order to provide optimal growth and protein utilization, diet with 50% of replacement of fish meal with SPP is recommended. The result that fingerling fed 25-75% SPP inclusion had better growth performance that the diets with a single animal protein source (single SPP or fishmeal) is not surprising because protein intake was higher for these diets. Protein intake of fish is the major determinant of fish growth. It was reported that a mixture of protein sources was much more effective than diets with single animal source of protein [15, 16, 17].

The investigation has shown that SPP was founded to be a viable alternative to fish meal replacement in african catfish fingerling feeds. SPP is very promising for Uzbekistan because that country has developed silk breeding. Silkworm pupa for itself is sub-product which can be utilized. One of promising ways of utilization is production of such high value product as chitozan [9] developed in last decades. Silkworm pupa protein is sub-products of chitozan production. Our investigations showed that this protein can be used in aquafeed, and such utilization will increases rationality of silkworm breeding and provide aquafeeds production with high quality source of animal protein.

5. Conclusion

From the result of our investigation we can conclude that silkworm pupa protein can be alternative source of replacement for fish meal in African catfish feed and up to 50 % replacement of fish meal with silkworm pupa protein advised.

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