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Dietary Inclusion of Superworm (*Zophobas morio*) Larvae for Asian Stinging Catfish (*Heteropneustes fossilis*) Culture: Impacts on Growth Performance

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ABSTRACT:
The present study aimed to find out the potential of superworm larvae as an alternate protein source in aquafeed for fishes. The feeding trial lasted for 45 days in glass aquariums, with four treatments (Control, T_1 T_2 and T_3) for stinging catfish (*Heteropneustes fossilis*). In control, fishes were fed without superworm larvae (SWM) while in different treatments (T_1, T_2, and T_3) fishes were fed with feed pellet containing 10%, 20%, and 30% processed superworm larvae respectively. The study showed that the growth of *H. fossilis* significantly improved with superworm larvae-based diet and maximum mean length 7.47±0.198 and weight 2.94±0.085 was observed in T_2 where fishmeal was replaced by superworm larvae (20%). The minimum length and weight gain by the fishes was in control (7.185 ±0.032 cm) and (1.77 ±0.049 g) respectively. Specific growth rate and protein efficiency ratio was maximum 1.95± 0.120 and 0.07±0.0 in T_2 respectively, whereas it was minimum 1.29 ± 0.028 and 0.04±0.0 for control respectively. The average daily weight gain of fishes from all treatments were found to be significantly different from control, and specific growth rate was significantly different (0.000) of all the treatments from control. The statistical calculation exhibited that the protein efficiency ratio in the experimental groups was not significantly different (0.299) from each other. It is suggested that the 20% superworm larvae contained sufficient amount of the protein which help for better growth.

Keywords:
Superworm, Alternative protein source, Food security, Stinging catfish

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INTRODUCTION

The fish feed industry's heavy reliance on fish meal has led to increased expenses in producing nutritionally balanced aqua feeds. The surge in demand for fish meal, particularly in the commercial fish diet industry, has led to a scarcity in supply, causing prices to rise. It is imperative to decrease the reliance on fish meal in fish diets by substituting it with alternative protein sources. This is necessary as relying solely on fish meal can have detrimental effects on the overall functioning of aquaculture systems, ultimately leading to a decrease in production (Jabir et al., 2011). Fish meal is frequently in short supply and costly due to its utilization in poultry feeds, as well as its unpredictable availability throughout the year (Gumus et al., 2009). The viability of plant protein sources as a substitution of fish meal but due to presence of anti-nutritional factors has been recognised as constraining the use (Mo et al., 2014; Cheng et al., 2016; Choi et al., 2016). Consequently, the consideration of replacing with alternative protein sources in fish feeds is crucial but due to significant nutritional value insects are increasingly being recognized as valuable feedstuffs in the aquaculture industry and considered as promising feedstuff for animals' diet (FAO, 2013). Insects are rich in protein with well-balanced amino acids, minerals, vitamins, fibre, bioactive compounds such as chitin and lauric acid, antimicrobial peptides, carotenoids and some species even contain high levels of polyunsaturated fatty acids like oleic and linoleic acids (Józefiak et al., 2019; Sogari et al., 2019 and Zadeh et al., 2019) therefore these are also constraining in use for fish meal. The fish meal substituted with alternative nutrients like superworm meal (SWM) to lower the overall cost of fish feed and balancing in nutrient supply.

The superworm larvae, *Zophobas morio* is the larvae of darkling beetle and belongs to family *Tenebrionidae* and found worldwide. Raising of these worms are quite simple and can grow on a substrate containing various organic wastes, such as vegetable and garden wastes. These are inherently larger than regular commercial mealworms (Hosseini et al., 2021) and use as feed for bird, reptile etc. due to their high nutritional value (Rumbos and Athanassiou, 2021). It is also reported that superworms are one of the important sources of fat and protein in poultry feed (Benzertiha et al., 2020). The fatty acid content ranges from 40% to 41% in dry matter, crude protein concentration of 44%–47% (Benzertiha et al., 2020; Jabir, 2012; Nederlof et al., 2017; Soon et al., 2018). In previous researches, superworms were considered as substantial fishmeal replacement in the diet of Nile tilapia (Jabir et al., 2012), juvenile rainbow trout (Shekarabi et al., 2021), Asian sea bass (Prachom et al., 2021), sea trout (Mikołajczak et al., 2020) and goldfish (Das et al., 2024).

Stinging catfish (*Heteropneustes fossilis*), is native food fish of India, is highly valued and widely cultivated in various Asian countries (Akand et al., 1991). It is highly nutritional fish and suggested by medical professionals as suitable diet for recovering patients (Alok et al., 1993) and also recommended for individuals who suffering from malaria (Bhuiyan, 1964). In West Bengal, this fish is in high demand during the summer months, predominantly sold (90%) in live condition (Fernado et al., 2019) and a small quantity is also exported for the aquarium trade (Gunasekara, 2011). But sufficient reports and documentations are not available on superworm meal as a fishmeal replacement for stinging catfish (*H. fossilis*).

Therefore, in present study feeding trial was undertaken to find out the effect of dietary inclusion of fishmeal protein by superworm larvae protein on growth performance and survivability of stinging catfish (*H. fossilis*) fingerlings.

MATERIALS AND METHODS

The experiment was carried out at Fish Biology Laboratory, Aquacultural Engineering Department, Indian Institute of Technology, Kharagpur (West Bengal).

The superworm larvae containing fish feed was prepared at Precision Aquaculture laboratory of the Aquaculture Engineering Department, IIT Kharagpur. The fresh superworms larvae were brought from the local pet shop and oven dried at 50.0 °C for 24 hours and dried superworms larvae were milled and stored at 8.0 °C for feed preparation (Mikołajczak et al., 2020). The software WinFeed (2.8) was used to formulate the 4 iso-nitrogenous (40% crude protein) diets.

All fish feed ingredients were grounded thoroughly to ensure the homogeneity and required amount of water added to make a dough and pelletizing by hand pelletizer (2.0 mm mesh sieve). The pellets were dried at 70.0 °C in hot air oven for 24 hr. to remove the moisture. They were packed in plastic bags, labelled and stored at room temperature in the laboratory. The fishes were fed @ 3% of fish body weight twice a day and the feeding rate was re-adjusted biweekly according to body weight of fish.
Fingerlings (240) of the Asian stinging catfish, *H. fossilis* (Bloch, 1796) with an average weight of 2.13±0.14 g were acquired from a freshwater hatchery Mecheda, Medinipur, West Bengal and were acclimatized at the laboratory for 10 days. The feeding trial was conducted in two replications for the duration of 45 days and each replication were stocked by 30 fingerlings. The control diet containing normal fish feed without superworm larvae and fish feed for diets with superworm larvae 10% (T_1), 20% (T_2) and 30% (T_3).

Proximate analysis
The proximate analysis of fish feed was analysed in the Precision Aquaculture Laboratory, Aquacultural Engineering Department, IIT Kharagpur to followed the procedure of Association of Official Analytical Chemists technique (AOAC, 2006).

Growth analysis
The growth of fish was expressed as Survival rate, weight gain (WG), percentage weight gain, average daily weight gain (ADWG) and specific growth rate (SGR), Nutrient utilization indices such as feed conversion ratio (FCR), and protein efficiency ratio (PER) were analysed as follows.
Fish survival rate (%) = F_2 / F_1 x 100
Where: F_1 is number of fish at beginning of the experiment and F_2 is number of fish at end of experiment.
Length gain (LG) = $L_2 - L_1$
Weight gain (WG) = $W_2 - W_1$
Where: W_2 & L_2 are mean of final weight and length W_1 & L_1 mean of initial weight and length
Average daily weight gain (ADWG) = Weight Gain (g) / time (Number of days)
Specific growth rate (SGR) = ($\ln W_2 - \ln W_1$ /T) x 100
Where: $\ln W_2$ is final weight of fish, $\ln W_1$ is initial weight of fish and T is days of experiment
Feed conversion ratio (FCR) = Feed offered / Weight gain
Protein efficiency ratio (PER) = Weight gain (g) / Protein fed (g).

Statistical analysis
Data analysis was performed by one-way analysis of variance (ANOVA) using SPSS version 12.0. The means variations were compared using Duncan's post hoc test at 5% probability level.

Monitoring of water quality
Physico-chemical parameters of fish tank water were analysed bi-weekly using Multiparameter (ProQuatro SKU606950).

RESULT

The proximate analysis of the fish feed was done and represented in table 1. Proximate analysis of experimental fish diet shows that crude protein (39.80±0.13) and fat content (14.03±0.02) were observed minimum in control diet whereas same was maximum in diet of T_1 (40.76±0.22) and T_3 (19.46±0.08) respectively. Moisture and ash contents were found minimum (0.43±0.00) and (4.4±0.04) in the diet of treatment tank T_1 and T_2 while maximum in the diet of T_3 (0.95±0.01) and control (7±0.05) respectively (Table 1).

Table 1: Proximate analysis of experimental fish diet

Parameters	Control	T_1	T_2	T_3
Protein	39.80±0.13	40.76±0.22	39.90±0.16	40.107±0.07
Fat	14.03±0.02	17.02±0.11	18.26±0.03	19.46±0.08
Moisture	0.53±0.02	0.43±0.00	0.73±0.02	0.95±0.01
Ash	7±0.05	6.4±0.03	4.4±0.05	5.03±0.08

All values are means ±SE.

The treatment cohorts exhibited a consistent 100% survival rate throughout the experiment and no discernible variations in survival rates across the treatments i.e. control, T_1, T_2 and T_3 (Table 2). The maximum length gain (7.47±0.1979) and length gain percentage (11.25±0.509) was observed in fish of T_2 tank whereas minimum length gain (7.185 ±0.032) length gain percentage (7.65±0.396) was observed in control (Table 2). The weight gain (2.94±0.085), weight gain percentage (142.1±13.29) and average daily weight gain (0.06± 0.00) was observed maximum in fishes of T_2, differing to this minimum weight gain (1.77±0.049), weight gain percentage (77.51±2.637) and average daily weight gain (0.035±0.007) was observed in control tank fishes (Table 2). The maximum feed conversion ratio (2.095 ±0.247) was observed in T_3, while T_2 (1.225±0.064) exhibit good feed conversion ratio. Specific growth rate and protein efficiency ratio was noted maximum (1.95±0.1202) and (0.07±0.0) respectively in T_2, while it was minimum (1.29 ±0.028) and (0.04±0.0) in control fish tank (Table 2).

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Table 2: Growth performance of studied fish fingerlings fed with the experimental diet

Growth parameter	Control	T_1	T_2	T_3
Survival rate (%)	100 ± 0.0	100 ±0.0	100 ±0.0	100 ±0.0
Length gain	7.185 ±0.032	7.281 ±0.005	7.47 ±0.1979	7.258 ±0.092
Length gain (%)	7.65±0.396	7.77±0.473	11.25±0.509	8.235 ±6.314
Weight gain	1.77 ±0.049	2.33 ±0.014	2.94 ±0.085	2.48 ±0.431
Weight gain (%)	77.51±2.637	121.6±28.09	142.1±13.29	115.5±24.72
Average daily weight gain	0.035±0.007	0.05 ± 0.00	0.06 ± 0.00	0.05 ± 0.00
Feed conversion ratio	1.755±0.035	1.35±0.191	1.225±0.064	2.095±0.247
Specific growth rate	1.29 ± 0.028	1.75±0.282	1.95± 0.1202	1.69±0.2545
Protein efficiency ratio	0.04±0.0	0.05 ±0.0	0.07 ±0.0	0.06±0.0

*All values are means ± SE

The growth parameters including length gain (cm), length gain (%), weight gain (gm), weight gain (%), average daily weight gain (gm), feed conversion ratio, specific growth rate and protein efficiency ratio were $T_2 > T_1 > T_3$ which indicating that growth performance of studied fish were significantly influenced by superworm larval (T_2, 20%) containing fish feed. The statistical analysis also indicated that average daily weight gain and specific growth rate of studied fishes were significantly different (p 0.01) in all the treatments compare to control while protein efficiency ratio of the experimental groups was not significantly different (p 0.01) from each other (Table 3 and 4).

Table 3: ANOVA for different group of studied fish

Parameters		Sum of Squares	Mean Square	F	Sig.
Length gain (cm)	Between groups	0.217	0.072	1.958	0.153
	Within groups	0.738	0.037		
	Total	0.955			
Length gain (%)	Between groups	48.062	16.021	1.790	0.181

	Within groups	178.990	8.949		
	Total	227.051			
Weight gain (gm)	Between groups	1.362	0.454	0.894	0.461
	Within groups	10.154	0.508		
	Total	11.516			
Weight gain (%)	Between groups	5623.740	1874.580	1.387	0.276
	Within groups	27021.693	1351.085		
	Total	32645.433			
Average daily weight gain	Between groups	0.002	0.001	85.000	0.0001
	Within groups	0.000	0.000		
	Total	0.002			
Feed conversion ratio	Between groups	3.176	1.059	3.287	0.042
	Within groups	6.442	0.322		
	Total	9.617			
Specific growth rate	Between groups	1.683	0.561	12.304	0.0001
	Within groups	0.912	0.046		
	Total	2.594			
Protein efficiency ratio	Between groups	0.001	0.000	1.309	0.299
	Within groups	0.007	0.000		
	Total	0.008			

Degree of freedom (*df*) between groups (3.0), within groups (20.0) and total (23)

Table 4: Multiple Comparisons (Tukey HSD) between various growth parameters

Parameter	**Treatment**		**Mean Difference**	**Sig.**
Length Gain	C	T_1	-0.017	0.999
		T_2	-0.240	0.167
		T_3	-0.101	0.801
	T_1	T_2	-0.223	0.216
		T_3	-0.084	0.874
	T_2	T_3	0.140	0.598
Length Gain (%)	C	T_1	-0.183	1.000
		T_2	-3.528	0.206
		T_3	-1.637	0.780
	T_1	T_2	-3.345	0.245
		T_3	-1.453	0.834
	T_2	T_3	1.892	0.696
Weight Gain (gm)	C	T_1	0.070	0.998
		T_2	-0.532	0.578
		T_3	-0.272	0.911
	T_1	T_2	-0.602	0.477
		T_3	-0.342	0.839
	T_2	T_3	0.260	0.920
Weight Gain (%)	C	T_1	-29.373	0.523
		T_2	-42.198	0.225
		T_3	-25.173	0.642
	T_1	T_2	-12.825	0.930
		T_3	4.200	0.997
	T_2	T_3	17.025	0.853
Average Daily Weight Gain	C	T_1	-0.015*	0.000
		T_2	-0.025*	0.000
		T_3	-0.015*	0.000
	T_1	T_2	-0.010*	0.000

		T_3	0.000	1.000
	T_2	T_3	0.010*	0.000
Feed conversion ratio	C	T_1	0.750	0.134
		T_2	0.970*	0.036
		T_3	0.447	0.536
	T_1	T_2	0.220	0.907
		T_3	-0.303	0.792
	T_2	T_3	-0.523	0.403
Specific growth rate	C	T_1	-0.485*	0.004
		T_2	-0.720*	0.000
		T_3	-0.522*	0.002
	T_1	T_2	-0.235	0.257
		T_3	-0.037	0.991
	T_2	T_3	0.198	0.396
Protein efficiency ratio	C	T_1	-0.005	0.964
		T_2	-0.018	0.332
		T_3	-0.015	0.501
	T_1	T_2	-0.013	0.596
		T_3	-0.010	0.780
	T_2	T_3	0.003	0.989

The studied water quality parameters i.e. water temperature (26.88 to 28.16 °C), dissolved oxygen (5.97 to 6.39 mg/l), pH (7.38 to 7.43), ammonia (0.02 to 0.03 mg/l) and nitrate (2.00 to 2.96 mg/l) were observed in all treatments during the study (Table 5). These findings show that the water quality in experimental tanks was optimum and not affected by the provided fish feed Gopalkrishnan et al, (2014).

Table 5: Water quality parameter (mean± SD) of different treatment tanks

Parameter	Control	T_1	T_2	T_3
Dissolved oxygen (mg/l)	6.24±0.51	6.39±0.44	6.16±0.40	5.97±0.98
pH	7.43±0.02	7.42±0.02	7.38±0.01	7.42±0.01
Ammonia (mg/l)	0.02±0.00	0.02±0.00	0.02±0.00	0.03±0.01
Nitrate (mg/l)	2.96±0.21	2.00±0.34	2.11±0.29	2.02±0.26
Temperature (°C)	27.05±1.02	26.88±1.10	27.06±0.44	28.16±0.99

*All values are means ± standard error

DISCUSSION

The survival and growth of *H. fossilis* influenced by the quality and quantity of protein in feed. Hence, it is crucial to explore sustainable protein sources for this commercially important fish species. Currently, larvae meals are promoted as natural alternative protein sources for fish nutrition as they contain high-quality protein and provide antimicrobial peptides, Gasco et al. (2018), Teles, (2012).

The present study represents the introduction of superworm larvae meal to *H. fossilis* diets at various percentage. The results are characterized by a high survival rate, satisfactory growth performance and feed utilization parameters. The positive effect of superworm larval meal was reported on Nile tilapia (*O. niloticus*) when 25% superworm larval fishmeal was given (Jabir et al, 2012). Tilami et al, (2020) also reported the better Specific growth rate in *Perca fluviatilis*, when fed with fishmeal containing 25% superworm larvae.

In this study, the growth of *H. fossilis* fish fed with superworm larvae diets at different percentage (T_1, T_2, and T_3) improved remarkably when compared with control, indicating that these feeds were able to meet the nutrient requirements of studied fish. The growth performance in stingray catfish was optimum in T_2 where 20% superworm larvae meal was incorporated. In agreement with present study Jabir et al., (2011) reported that 25% superworm meal inclusion in the diet of Nile Tilapia contributed the notable result. Hosseini et al., (2021) also advocated the 20% inclusion of superworm meal in fish feed of fingerling rainbow trout. However, in present study when the replacement level reached up to 30% (T_3), the growth was hindered, as indicated by the reduced weight gain percentage, specific growth rate, and final weight gain. The author suggests that the decrease in maximum weight gain, specific growth rate, feed conversion ratio and protein efficiency ratio can be attributed to the increased levels of chitin in the full-fat super worm larvae meal. Chitin is not easily digestible in monogastric animals, and a higher consumption of chitin can lead to a reduction in growth. This is because chitin reduces the digestibility of certain macronutrients by decreasing the retention time of feed in the intestine and limiting the accessibility of enzymes to the feed substrates (Bruni et al., 2018; Fontes et al., 2019; Magalhães et al., 2017; Makkar et al., 2014; Xiao et al., 2018). Differing to this Das et al., (2024) reported that maximum growth performance, skin colouration, and survival rate of goldfish was achieved when 42.35% fish meal substituted by swperworm larvae. Similarly, Prachom et al., (2021) also noted that replacing fishmeal with superworm meal at 80% had the highest growth efficiency and feed utilization in Asian sea bass, *Lates calcarifer*. The growth of fish is influenced by various factors, including size variations and the interaction of environmental factors such as water temperature, competition among individuals, feed quality, age, and maturity (Moyle and Cech, 2011). These factors play a significant role in determining the growth rate of fish. Additionally, (Ghaly and Alkoaik, 2009) found that fish require essential amino acids for optimal growth, which can be obtained from mealworms.

Water quality parameters for the rearing of *H. fossilis* larvae during the experiment were in the acceptable range as mentioned by Rahman et al., (1982). Similar and optimum water quality range for rearing *H. fossilis* larvae was reported from Bangladesh Ahmed et al., (2016). The volume of insect meals is currently still minimal, but is increasing. The inclusion of larval meals remains insignificant in compared to other components in aquafeeds. The insect larval farming industry possibly to flourish in the next decade. It is certain that in the near future larval based fish feed ingredient will affect aquaculture considerably and make aquaculture green, profitable and sustainable.

CONCLUSION

The alternative protein source, superworm meals could be one of the finest options as fish meal replacement in aquafeed for commercially important fish species. Investigation revealed that inclusion of 20% superworm meal in fish feed of the juvenile of *H. fossilis* support growth performance, feed conversion, Protein efficiency and Survival rate. Further research is needed to explore the optimal inclusion rate and the long-term effects of super worm larval meal on fish health and performance.

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Author Contributions
"All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mr. Tirthankar Shah under the observation and supervision of Dr. G.D.Bhowmick and Arka Pramanik. The first draft of the manuscript was written by Dr. Nandita Deshpande and Dr. N.C. Ujjania contributed by editing and refining the manuscript. All authors read and approved the final manuscript."

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