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ALTERNATIVE PROTEIN SOURCE FOR AQUAFEED PRODUCTION FOR CATFISH, *PANGASIUS HYPOTHALAMUS* (SAUVAGE, 1878)

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Abstract

Silkworm pupae, a waste of the silk industry, are produced in large quantities in Kayin State and Mandalay region of Myanmar and are often discarded to the surrounding environment or used as a fertilizer for plant growth. In the present study, silkworm pupae were used as an alternative animal protein to replace fishmeal used as experimental feed for catfish, *Pangasius hypothalamus*. The feeding trial had three treatments: (1) silkworm pellet (SWP) with 26% of crude protein; (2) commercial floating pellet (CFP) with 32% protein, and (3) commercial sinking pellet (CSP) with 28% protein. Silkworm pellet (SWP) was formulated and produced using a small extruder in the laboratory. Total 180 fingerlings of catfish divided into six groups were stocked in glass aquariums providing two replication tanks for each treatment. The fish were cultured for 3 months from October to December 2019 in the laboratory condition. Among the three different diets, the highest growth rate was observed in CFP with (3.9 %/ day) and the second highest growth rate was recorded in SWP with (3.7 %/ day). Feed Conversion Ratio (FCR) of SWP, CFP and CSP were 3.37, 2.8 and 4.05 respectively. The weight gains (WG) of CFP, SWP and CSP were 10.57, 9.36 and 8.43, respectively at the end of experiment. Only 3% of mortality was found in the tank treated with CFP. This study revealed that the silkworm had very high protein content (52%) and it could be considered as an alternative dietary supplement for catfish.

Keywords silkworm pellet, floating pellet, sinking pellet, *Pangasius hypothalamus*

Introduction

In the compound fish feed, fish meal is primarily used as a protein supplement (Fisheries Centre Research Reports, 2016). About 56% of fish meal was used to feed farmed fish, 20% was used in pig feed, 12% in poultry feed, and 12% in other uses, which included fertilizer. (Miles *et al*., 2015).

Fishmeal can be prepared from almost any kind of seafood, but is generally produced from wild-caught, small marine fish that comprise a high ratio of bones and oil, and are usually supposed to be not appropriate for direct consumption of human. The fish caught for fishmeal drives exclusively are termed "industrial". Fish meal was produced from by catch and byproducts of extras made during processing of various seafood products intended for human consumption (Miles *et al*., 2015).

Small fish will be lost in the trophic level because of high demand of fishmeal manufactured so we need to maintain small fish for sustainability in natural water bodies in Myanmar. Using cultured insect meal instead of fishmeal in the preparation of fish feed is one of the important requirement to develop the sustainable aquaculture. Nowadays, potential inset meals have been considered to be used in aquaculture sector.

Sericulture has been accomplished in many developed and developing countries in the world for many years ago. Among those countries, Japan, Korea, Brazi, China, Thailand, India, Turkey, Iran, and Myanmar were well-known silk industry (Barber,1992). Since 7th century, sericulture was started and recent sericulture was known in 1952 (Barber, 1992). In Myanmar, silkworm (*Bombyx mori*) rearing has been practiced from the earliest time in rural zone and it is still completed as a cottage type in a traditional way. The spent pupae are produced in large quantities and are a major by-product in silk production (Datta, 2007). Eight kilogram (8 kg) of wet pupae can produce one kilogram (1 kg) of raw silk (Patil *et al*., 2013). Pupae are waste material often rejected in the open environment or used as fertilizer in silk production in

Karen State and Mandalay Division in Myanmar. It can be used as one of the ingredients for making fish feed.

The by-product pupa has high nutrition level especially for the source of protein (Hiroyuki *et.,al*, 2010). If we can substitutes by-product pupae in aqua feed production, the natural fish resource can be managed in terms of sustainable fisheries production.

In the present study, the nutritional value of spent pupae was studied and aimed to understand basic nutritional value of by-product**.** Then, aqua feed for catfish was produced using by pupa.

Materials and Methods

Study site and study period

The experiment was conducted in the Aquatic Bioscience Laboratory at the University of Yangon in Myanmar for six months during September 2019 - February 2020.

Experimental fish and design of fish tank

Catfish (*Pangasius hypothalamus*) (Sauvage, 1878) was selected for the present study. A total of 180 fingerlings of the same batch (BW 7.6±1.58g) were purchased from the hatchery of Department of Fisheries at Hlawgar in Yangon Region. The fish were acclimatized into laboratory condition for one week before the experiment and divided into six groups.

Six glass tanks (1m x 0.5m x 0.5m) with 122 liters/tank of water volume were prepared and set up aeration system to maintain dissolved oxygen in the tanks before experiment. All of the fish were starved for 24 hours before the experiment. After 24 hours, random collected 30 fingerlings were weighed and measured for initial data, which were then stocked in each glass tank (Plate. 1). The experimental feed were introduced into the study fish tank with 5% of body weight and two times per day (10:00 AM and 4:00 PM). The water exchange was done with two days interval and water quality parameters such as temperature, pH, dissolved oxygen and ammonia were analyzed on initial day and subsequently at fortnightly interval by standard methods. The fishes in all treatment were measured and recorded the weight after 30 days intervals to analyze the growth performance of the study fish.

Analysis of nutritional value

The experimental fish feed was prepared before the experiment. The spent pupae were dried in oven with temperature (100 ºC). Dried pupae were grinded into powder and their nutritional values such as crude protein, fat, carbohydrate, moisture, ash and fiber were measured at the laboratory in UMFCCI (Union of Myanmar Federation of Chambers of Commerce and Industry) (Table 1).

Preparation of silkworm feed

The silk worm pallet was produced based on the feed formula for catfish. All ingredients (silkworm powder, wheat flour, cassava, vitamin C, carboxymethyl cellulose, butylated hydroxytoluene, guar gum and fish oil) were mixed by the ratio of protein percentage for this experiment (Table 2). Silkworm pellet was produced by a handmade pellet machine (Plate 1). Three different kinds of experimental feed such as silkworm pellet (SWP) with 26% of protein, Floating pellet (CFP) with 32% protein, sinking pellet for control feed (CSP) with 28% protein. Two replications for each experiment were conducted simultaneously.

Growth Parameters and data analysis

The growth performance of experimental fish weight gain (WG), specific growth rate (SGR), percent weight gain (PWG), feed conversion ratio (FCR) and gross conversion efficiency were calculated according to the following formulas.

Weight Gain

The body weight of *Pangasius hypothalamus* (Sauvage, 1878) fingerling was obtained initially and thereafter at thirty days interval up to completion of the experiment i.e. 60th days.

The weight gain (g) was calculated as given below:

Weight gain (WG)

Final weight (g) - Initial weight (g)

Specific Growth Rate (SGR)

$$\text{SGR (\%)} = \frac{\text{Ln (final weight in grams)} - \text{Ln (initial weight in grams)}}{\text{Experimental days}} \times 100$$

Percent weight gain (PWG)

$$\text{PWG (\%)} = \frac{\text{final weight (g)} - \text{initial weight (g)}}{\text{Initial mean weight (g)}} \times 100$$

Feed Conversion Ratio (FCR)

$$\text{FCR (\%)} = \frac{\text{Weight of food intake (g)}}{\text{Weight gain of fish (g)}}$$

Table 1 Laboratory analysis of nutritional value of silkworm powder

Test Parameter	Test Method	(%) of compound
Moisture	AOAC-2000(930.15)	7.27
Ash	AOAC-2000(942.05)	4.04
Crude Protein	AOAC-2000(920.152) (Kjeldahl Method)	52
Crude Fibre	AOAC-2000(978.10) Fiber Cap Method	0.83
Crude fat (Ether Extract)	AOAC(Buchi Soxhlet Method)	29.62
Carbohydrate	By Difference	6.24
Energy value (kcal/100g)		502

Table 2 Experimental feed ingredients with silkworm powder meal

Ingredients	Percent of composition (%)
Silkworm	39.49
Wheat flour	48.26
Cassava	7.4
Vitamin C	2
Carboxymethyl cellulose(CMC)	0.33
Butylated Hydroxytoluene (BHT)	0.02
Guar gum	0.5
Fish oil	2


(A) Measuring of fish


(B) Weighing of fish


(C) Experimental tank


(D) Dried Silkworm


(E) Powdered silkworm


(F) Preparation of silkworm paste


(G) Extruding of fish pellet


(H) Cutting of pellet


(I) Silk worm fish pellet

Plate 1 Study species and making fish feed pellet using silkworm

Results

Preparation of formulated fishmeal

The proximate composition of silkworm pupae was shown in Table 1 and it contained 29.62, 0.83, 7.27, 4.04, and 6.24 of crude protein, crude fat, crude fiber, ash and carbohydrate respectively. The energy content was estimated to be 5.02 kcal /g.

The composition of experimental feed ingredients was shown in Table 2. The silkworm fish feed (SWP) was formulated with 26% of protein. The protein contents of CFP (commercial floating pellet) and CSP (commercial sinking pellet) were 32% and 28%, respectively.

Different diet comparison of SGR and FCR

The data on specific growth rate, survival and feed conversion ratio are presented in Table 3. Among three different diets, the highest growth rate was observed in floating pellet (CFP) with SGR (3.9±0.11). Feed Conversion Ratio (FCR) was recorded as (2.84±0.36) from two replications. The lowest growth rate of this experiment was observed in commercial sinking pellet with SGR (3.5±0.15%), the feed conversion ratio FCR was recorded as (4.05±0.95) from two replications. (Table 3)

Table 3 Mean initial body weight, FCR and SGR of three different diets

	SWP	**CFP**	**CSP**
Initial weight	8.05 ± 0.63	7.2 ± 0.56	8.5 ± 1.69
Final weight	17.41 ± 0.57	17.77 ± 0.16	16.93 ± 0.89
SGR	3.72 ± 0.007	3.9 ± 0.11	3.5 ± 0.15
WG	9.36 ± 0.05	10.57 ± 0.73	8.43 ± 0.8
FCR	3.37 ± 0.16	2.84 ± 0.36	4.05 ± 0.95
PWG	116.66 ± 9.9	147.66 ± 21.8	102.15 ± 29.8
Survival rate (%)	100	97	100

Note: SWP = Silkworm pellet, CFP= Commercial floating pellet,
CSP= Commercial sinking pellet

In the experiments, highest and second highest weight gains were resulted in fish treated with commercial floating pellet and silkworm pellet respectively. The lowest weight gain was found in control (commercial sinking pellet) (Figure 1).

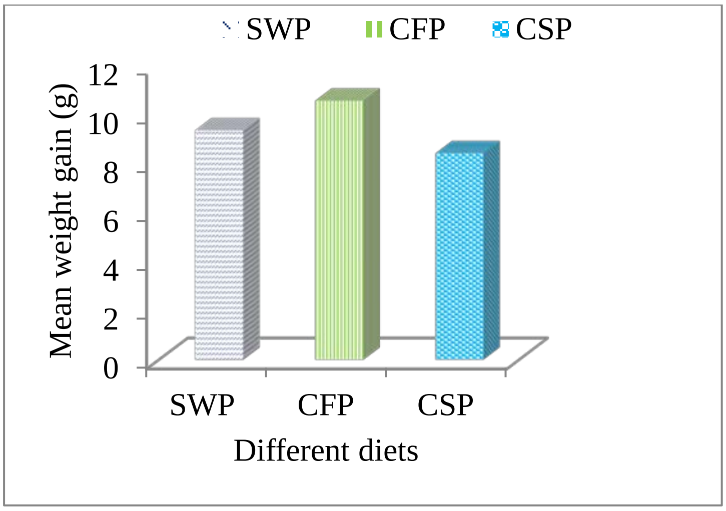

Figure 1 Mean weight gain of three different diets

The weight of fish in all experiments gradually increased during the study period. In the end of experiment, the weight of fish was 17.41±0.57, 17.77±0.16 and 16.93±0.89 in silkworm pellet (SWP), commercial floating pallet (CFP) and commercial sinking pellet (CSP) respectively (Figure 2).

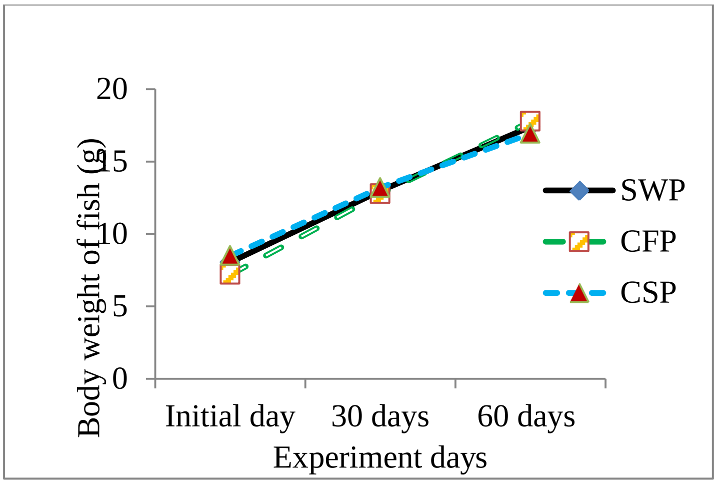

Figure 2 Comparison of mean initial and final weights

The lowest FCR values with the highest SGR % were recorded in fish feed with commercial floating pellet (CFP). The highest FCR values with lowest SGR % were recorded in fish feed with commercial sinking pellet (CSP). The weight of fish fed with the silkworm pellet (SWP) was followed by that of the commercial floating pellet (Figure 3 & 4).

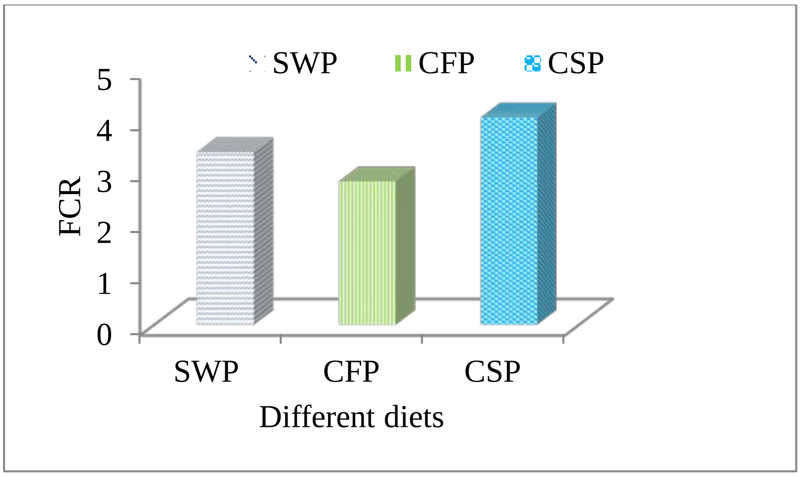

Figure 3 Comparison of feed conversion ratio (FCR) of three different diets

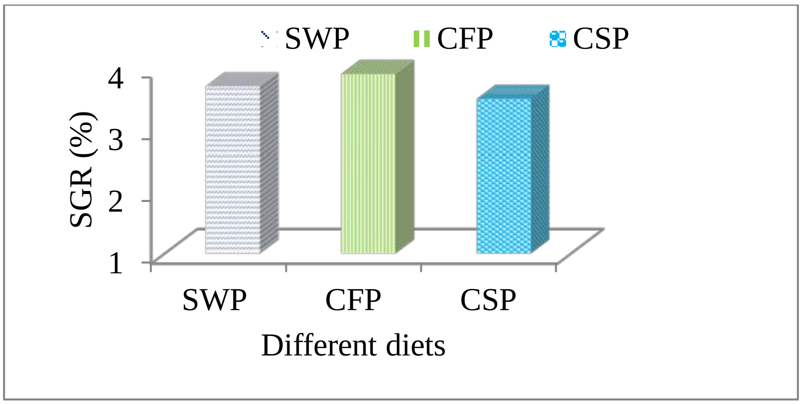

Figure 4 Comparison of specific growth rate % (SGR) of three different diets

Survival rates of three different diets were not differ during the study period except 97% in replicate 1 of commercial floating pellet as shown in table (4).

Table 4 Survival rate of each tank with three different diets during the study period

Different feed	Number of fish (initial)	Number of fish (30days)	Number of fish (60days)	Survival rate (%)
SWP	30	30	30	100
CFP	30	30	29	97
CSP(control)	30	30	30	100

During the experiment, pH, DO, Ammonia and temperature were recorded fortnightly and water exchange had regularly done two times per week. The results of water parameter in the tanks were shown in table (5).

Table 5 Water parameter range of three different diets during the study period

Different diets	Ammonia	pH	DO	Temp
SWP	0.2-0.6	5.5-6.5	6 - 7	22-23.8
CFP	0.6-1	5.5-6.5	6 - 7	22-23.8
CSP (control)	0.6-1	5.5-6.5	6 - 7	22-23.8

Discussion

Aquaculture is the fastest increasing area of world human food production and has a yearly increase of about 10% (FAO, 1997). The use of insects as a source of protein for fish nutrition was a relatively new approach (Bondari and Sheppard, 1981). Various development stages of insects have been used to feed fish and farm animals. Hickling (1962) noted that silkworm pupae have been an important component of carp diet in Japan and China (Newton *et al*., 1977). Therefore, efficient feed formulation should be made by utilizing the knowledge on the nutritional requirements and availability of local feed ingredients, diet palatability, acceptability and digestibility capacity of fish.

During the study period, the three different diets were used in the experiments. This study found that all treatments had increasing weight gain throughout the duration of the experiment. That proved that the catfish reacted favorably to all of the diets. Among them, fish fed commercial floating pellet containing 32% of protein had the highest growth and weight gain. The silkworm pellet containing 26% of protein had the second highest growth rate and the lowest growth rate 28% of protein content. The experimental fish feed with silkworm as the sole animal protein source contained only 26% crude protein. However, growth and FCR was comparable to commercial feeds containing either 32 or 28% crude protein. In contrast, previous research studies with different fish species recommend dietary inclusion levels of silkworm pupae meal in the diet as follows: 30-50% for major and minor carps, 5-15% for trout, 50-60% for masher, 75-100% for catfish, 30-40% for ornamental fishes and 5-20% for shellfishes. These inclusions assured to give better growth performance compared to sole fishmeal as the protein source.

During the study period, the silkworm pupae diet we employed in our research contained only 26% protein. Abdullo *et al.* (2015) studied that effect of the replacement of fish meal with silkworm pupa protein on the growth of *Clarias gariepinus* fingerlings, and concluded that a 50:50 ratio of silkworm pupae and fish meal was appropriate. The authors studied that the growth rate was higher in the group fed diets containing a mixture of fishmeal and silkworm pupae, while it was lower in the group fed diets containing only fishmeal or silkworm pupae at 100%. These findings demonstrated that feeding silkworm pupae in part place of fishmeal resulted in higher growth performance than feeding fishmeal alone. According to Faturati *et al.* (1986), and Akiwanda *et al*. (2002), feed with a protein concentration of 39–41% is optimal for feeding catfish. So, the current findings were insufficient to justify replacing fishmeal with silkworm pupa protein in the diet.

It is advised to replace 50% of the fish meal in the diet with silkworm pupae in order to promote appropriate growth and protein utilization. Our research revealed that this protein can be used in aquafeed, and that doing so will improve the rationality of silkworm rearing and supply high-quality sources of animal protein for aquafeed production. The findings of our experiment point out that silkworm pupa protein can be used as an alternative to fish meal in the diet of catfish.

Conclusion

Insect meal is one of the best protein sources for partial or total replacement of fishmeal in aquaculture feeds. This is mainly due to good amino acid and fatty acid profiles. Moreover, insects are natural food sources for fish. The present study revealed that, the SWP was very rich source of proteins, lipids and minerals so could be used as an alternative dietary supplement in fish feed. According to the experiment, fish fed with silkworm pellet had a comparable growth rate, and relatively low FCR was found by the control (sinking pellet). Therefore, silkworm pellet was suitable for fish culture to reduce feed cost and increase yield and income. When silkworm pupae are locally available, fish farmers can utilize the pupae as an excellent local feed ingredient to replace fishmeal in the pelleted feed and reduce feed cost.

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