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Effects of replacing dietary shrimp meal and soybean meal with silkworm (*Bombyx mori*) pupae meal on growth performance of rainbow trout *Oncorhynchus mykiss*

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Abstract
Shrimp and soybean meal are important and expensive protein sources for rainbow trout (*Oncorhynchus mykiss*) in Nepal. During the 90-day trial, this study examined the effects of replacing shrimp meal (SM) and full-fat soybean meal (FFS) with varied levels of spent silkworm (*Bombyx mori*) pupae meal (SPM) on growth performance, feed conversion ratio, and survival. Four treatment groups were examined for the experiment: T_1: 0% SPM, T_2: 12.5% SPM, T_3: 25% SPM, and T_4: 37.5% SPM. Three replicates of 125 fish (2.5±0.2 g) were used for each treatment group. As SPM rose in the diet, final weight, weight gain, specific growth rates, and daily growth rates increased significantly ($p<0.05$). Experimental diets had no effects on condition factors, feed conversion ratios, and survival. In conclusion, SPM can replace SM and FFS up to 35.7% in rainbow trout diets without affecting growth and feed utilization.

Keywords: Silkworm, insect meal, black soldier fly, flow-through system

1. Introduction

Crustaceans (SM) is one of the major feed ingredients used to prepare the feed for rainbow trout (*Oncorhynchus mykiss)* in Nepal [1], perhaps since crustaceans contain a large amount of astaxanthin, which has been shown to increase the natural red pigmentation of salmonids [2]. Rainbow trout is the only successfully cultured species on the hills and mid-hills of Nepal at the high stocking densities (75-100 fish per square meter) in the flow-through system [3]. Despite its slow-growing nature (200-300 grams per year) in the context of Nepal [4], it is the most produced species in the hills of Nepal, contributing to the productivity enhancement of the hill and mountain agricultural sectors [5-7]. Moreover, rainbow trout farming offers substantial opportunities for food and nutrition security in the hills and mountains through job and income opportunities [7]. Although rainbow trout farming is the fastest-growing aqua-cultural sector in the hills of Nepal and has been distinctly visible in terms of fish production and increasing income flow to rural hills in urban areas in wider parts of the Himalayan highlands [8], its sustenance and expansion have become a challenge for farmers because the major ingredient, shrimp meal, which is included up to 50% in the trout feed [1], is becoming scarce and expansive with the worldwide decline in fishery product [9]. In fact, it has become the main reason for high farmer-level commodity prices in response to the liquidity of the fish market. As a result, it has become of utmost importance to search for alternative animal protein from non-conventional sources that can substitute conventional sources, thereby reducing the cost of feed. However, the availability of quality dried shrimp and fluctuating prices have greatly affected the sustainability of trout farming. Therefore, the replacement of shrimp meal with locally available and cheaper animal protein feed ingredients is likely to contribute to reducing the costs of rainbow trout feed and help the expansion of the trout industry in Nepal.

The nutritional composition of silkworm pupae has attracted interest and has been identified as a viable option for feeding livestock as an alternative protein source, particularly monogastric species such as poultry, pigs, and fish, as well as ruminants [10, 11]. A study has shown that defatted SPM can be incorporated into the feed of cattle up to 30% without affecting nutrient utilization while replacing soybean meal [12]. Non-deoiled silkworm pupae, when incorporated into the feed of Catle (*Catla catla*) at 30%, showed higher growth compared to the control group [30]. Similarly, with Rohu fingerlings (*Labeo rohita*), SPM has been shown to replace fish meal (FM) by 50% while improving feed utilization and growth performance [14]. As such, SPM, which is a by-product waste of reeled silk, seemed to have a great potential for replacing many conventional protein sources due to its high nutritional value (crude protein: 50–70% and crude lipid: 24-33%) [15]. Though there are few studies on the feed formulation and nutrition of rainbow trout of table size [16], there is currently a lack of research on the substitution of shrimp meal (SM) with SPM in the diets of finger-sized rainbow trout (*Oncorhynchus mykisss*).

In addition to its potential to mitigate environmental pollution caused by the disposal of spent silkworm pupae, a feeding trial was conducted to investigate the impacts of partially substituting SM and soybean meal with SPM on the growth performance, feed utilization, and survival of rainbow trout fingerlings. Thus, the aim of this study was to develop cost-effective, high-quality feeds using locally sourced ingredients for the context of Nepal.

2. Materials and Methods

2.1. Experimental diets

The formulation of the experimental diets is given in Table 1. A basal diet was formulated using shrimp meal (SM) and full-fat soybean (FFS) as the major sources of protein and labeled as SM-based diet or T_1. The other three experimental diets were formulated by replacing 12.5%, 25%, and 37.5% of the main protein sources (SM and FFS) in an equal ratio with Spent Silkworm Pupae Meal (SPM) and labeled as T_2, T_3, and T_4, respectively. All the diets were formulated to be iso-nitrogenous (42% crude protein) using an Excel tool based on linear programming functionality called Solve in order to meet the minimum requirements of rainbow trout. In addition, the cost of each ingredient was also taken into account during feed formulation, as it is crucial to obtain the least cost per unit output and sustainability in terms of feed conversion efficiency.

Table 1: Formulation of experimental diets (% dry matter).

Feed Ingredients	T_1	T_2	T_3	T_4
Shrimp meal (SM)	35	30.63	26.25	21.88
Full fat Soyabean (FFS)	34.45	30.14	25.84	21.53
Spent Silkworm Pupae meal (SPM)	0	8.68	17.36	26.04
Soyabean oil (SO)	6	6	6	6
Wheat flour (WF)	15	15	15	15
White pea (WP)	5.5	5.5	5.5	5.5
Slack	2.55	2.55	2.55	2.55
Sisnu leaf powder (SLP)	1.5	1.5	1.5	1.5
Total (%)	100	100	100	100

Slack includes Sequivit-K (Vitamin premix) 1%, Aminomin (Mineral mix) 0.5%, Peezyme (multienzyme) 0.05 %, and Salt 1 % mixed per 100 kg of diet prepared

All the ingredients used were finely ground mechanically; the powdered ingredients were weighed and mixed thoroughly, followed by the addition of an appropriate amount of oil and water to produce pellets of good consistency. The prepared pellets were dried overnight in a dry oven at 40 °C and stored in airtight containers until use. Proximate analysis of the prepared experimental diets and some ingredients was done following the standard protocol of the Analysis of the Office Association of Chemists (AOAC, 1990) at the National Animal Nutrition Research Centre, Khumaltar, Lalitpur. The results of the proximate analysis are given in Table 2.

Table 2: Proximate composition (%) of experimental diets and some ingredients used.

Name of Sample	Dry matter	Ash	Crude protein	Crude fat
Diet 1 (T_1)	96.10	10.91	37.63	4.95
Diet 2 (T_2)	96.45	8.81	42.76	5.94
Diet 3 (T_3)	96.18	9.61	44.15	6.84
Diet 4 (T_4)	96.32	9.19	41.36	6.60
Sisnu powder	89.39	20.07	27.99	10.54
Silkworm pupa powder	93.32	5.24	56.28	11.55
White pea flour	93.69	3.54	32.52	8.16
Shrimp meal	92.67	8.62	58.51	6.28

2.2. Fish and rearing conditions

This study was performed at the Fishery Research Station (FRS), Trishuli Nuwakot. Rainbow trout fingerlings were brought from a farmer in Sindhupal Chowk district and acclimatized to the rearing conditions for one week at FRS. Fish were fed a basal diet by hand up to satiation three times a day while acclimating. After acclimatization, 1500 apparently healthy fish weighing 2.5±0.2 g were salt-bathed and randomly distributed into 12 nursing raceway tanks (1.25 m^2) at a stocking density of 125 fish per tank. The fish were divided into four experimental groups as follows: fish fed basal diet (T_1), fish fed 12.5% SM and FFS replaced diet (T_2), fish fed 25% SM and FFS replaced diet (T_3), and fish fed 37.5% SM and FFS replaced diet (T_4). These treatments were divided into three replicates. The fingerlings were fed one of the four experimental diets at a rate of 5% of body weight three times a day (08:00, 12:00, and 16:00) for 90 days. Any uneaten feed was collected 1-2 hours after feeding, dried, and weighed to estimate feed intake. Tanks were siphoned and scrubbed clean to remove feces and dirt when needed.

The source of water was the nearby river, from which water was collected in the siltation tank and then directed into the experimental tanks, maintaining a depth of 50 cm and a flow rate of 10 liters per second. The water quality parameters were measured daily, which ranged from 17.99±0.72 °C for temperature, 7.44±0.29 dissolved oxygen, and pH 7.47±0.03.

2.3. Sampling of fish and growth performance
Ten percent of the total fish from each tank were sampled fortnightly to estimate the amount of ration needed. Fish were starved for 24 hours prior to each sampling. At the end of the feeding trail, tanks were completely drained, and 10% of the fish were sampled for their length and weight, while the rest of the fish were used to estimate the batch weight per tank. The total number of fish that survived in each tank was also recorded at every sampling. Growth factors: final body weight (FW), weight gain (WG), daily weight gain (DWG), specific growth rate (SGR), and condition factor (K) were calculated based on the following formulae:

WG (g) = final weight (g) – initial weight (g)

DWG (g) = WG (g)/duration of culture (day)

SGR (%) = 100 × [In (final weight) – In (initial weight)]/days

$K = 100 \times [\text{final weight}/(\text{final length})^3]$

2.4. Statistical analysis
All the data (mean ±SEM) were arranged in Microsoft Excel and analyzed using SPSS software (Version 19, SPSS Inc., Chicago, IL, USA). Data were checked for normality and homogeneity of variance with the Shapiro-Wilk and Levene tests, respectively. All the data were subjected to a one-way ANOVA. A Duncan multiple range test was used when significant differences ($p<0.05$) were observed to rank the groups.

3. Results
Mortality in this experiment ranged from 44 to 82%, with the highest mortality observed in the control group. However, this phenomenon was not significantly affected by experimental diets. Figure 1 shows the growth trend of the trout fingerlings fed on four experimental diets over 90 days. Fish fed with the diets replacing 12.5%, 25%, and 37.5% SM and FFS with SPM showed significantly ($p < 0.05$) better growth than that on the control (T_1) (100% dietary protein as SM and FFS). The effect of spent silkworm pupae meal as a partial replacement of shrimp meal and full-fat soybean meal on the growth performance of rainbow trout fingerlings is presented in Table 3. The significantly highest weight gain ($p< 0.05$) of 13.8±0.72 g was obtained in T_3, when SM and FFS were replaced at 25% by SPM. The daily growth rate was also significantly highest (0.15±0.01 g) in T_3, whereas the significantly lowest daily growth rate (0.08±0.02 g) was obtained in T_1. The SGR of the fish in this experiment was more or less statistically similar, ranging from 1.40 to 2.01, with the lowest and highest values in T_1 and T_2, respectively. The FCR in this experiment did not vary significantly between diets ($p> 0.05$) and ranged from the lower of 1.25 in T_2 to the higher of 1.63 in T_1. No significant difference was found between the values of the condition factor for different experimental diets ($p> 0.05$).

The biweekly growth trend of rainbow trout fingerlings during the experimental period is shown in Figure 1. The fires grew moderately until 45 days of culture, steadily until 60 days, and exponentially from 60 to 90 days. At harvest, final body weight (g) was observed to be lower in treatment groups T_1 (9.4±1.37) and higher in T_3 (16.63±0.60), while other treatment groups T_2 and T_4 lay in between (Figure 1).

Table 3: Summary of growth performance of rainbow trout fingerling in different treatments in 90 days (Mean± SEM)

Treatment	Final Weight (g)	Weight gain (g)	Condition factor (K)	Daily growth rate, (gpd)	SGR	FCR
T_1	$9.4±1.37^a$	$6.8±1.40^a$	1.4±0.10	$0.08±0.02^a$	$1.40±0.17^a$	1.63±0.21
T_2	$15.33±0.42^{bc}$	$12.8±0.43^{bc}$	1.4±0.02	$0.14±0.01^{bc}$	$2.01±0.05^b$	1.25±0.01
T_3	$16.63±0.6^c$	$13.8±0.72^c$	1.5±0.11	$0.15±0.01^c$	$1.97±0.10^b$	1.27±0.03
T_4	$12.82±0.30^b$	$10.2±0.40^b$	1.3±0.11	$0.11±0.01^b$	$1.8±0.09^{ab}$	1.39±0.05

Values in each column with different superscript letters are significantly different at $p< 0.05$ (mean ±standard error of mean).

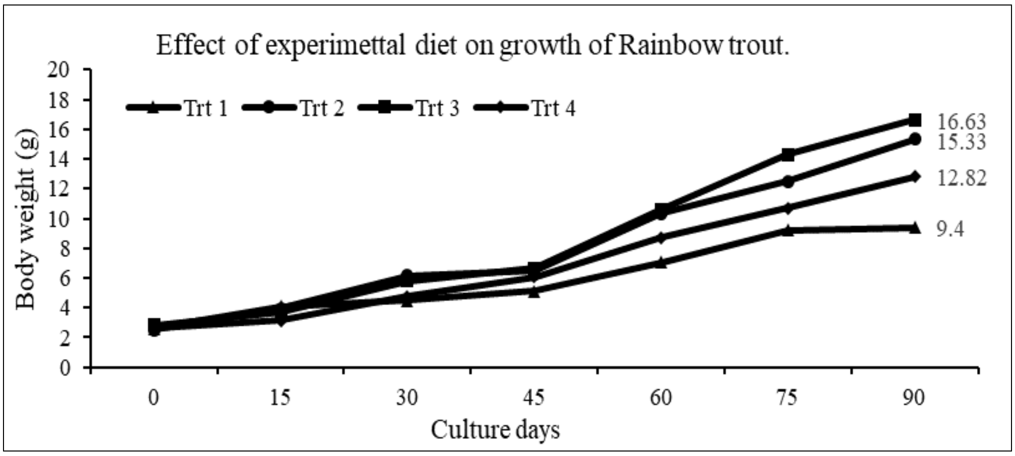

Fig 1: Growth trends of rainbow trout fingerlings in different treatments during an experimental period of 90 days.

4. Discussions
The utilization of silkworm meal as a feed ingredient for both monogastric and ruminant animals has been a longstanding practice in several Asian countries. The inclusion of silkworm pupae in fish diets has been found to promote optimal growth and enhance the fish's ability to resist infections [17].

In this study, we investigated the effect on growth, feed utilization, and survival of rainbow trout when SPM was used to replace shrimp and soybean meals. According to the results, growth was progressive in all the treatments during the period of the experiment, and survival was not affected. In support of our initial hypothesis that feed replaced with spent silkworm pupae meal (SPM) will not produce significantly lower growth performance than shrimp and soybean-based (control) feed, it was indicated via weight gain that rainbow trout responded positively to the partial replacement of shrimp meal and soybean meal with SPM. Several studies have shown that insect meal can be utilized to replace fishmeal in aquaculture at varied inclusion levels [18-22]. However, research on SPM as a substitute for shrimp and soybean meal in rainbow trout diets is still sparse. In this study, our results showed that up to 37.5% of the major protein source (shrimp and soybean meal) can be replaced by SPM without any adverse effects on growth, condition factor, or FCR, in contrast to the findings of Shakoori *et al.* [23], who reported reduced growth and increased FCR at more than 10% replacement of fishmeal in rainbow trout. In the current study, the survival of rainbow trout was not affected by the diets but presumably due to the turbid water with a high level of silt, resulting in skin erosion followed by pathogen infections within one month of stocking. Otherwise, SPM has been shown to be a valuable alternative to shrimp and soyabean meal replacement in rainbow trout feed up to a quantity of 37.5% while meeting the requirements of the rainbow trout for appropriate growth. In line with the findings of this study, Dheke and Gbhaju [1] reported that the growth response and feed utilization of rainbow trout fry were significantly improved during the eight weeks of feeding trail on the substitution of shrimp meal by SPM. It is probably because insect meals, including silkworm pupa meal, contain an appropriate ratio of essential amino acids, fatty acids, vitamins, minerals, and several antioxidant compounds known to promote fish growth and immunity [24]. However, their incorporation above 50% in the diet of several fish species has been reported to adversely affect growth physiology and feed utilization. For instance, Ji *et al.* [25] demonstrated that silkworm pupae meal can replace fishmeal up to 50% without having a significant impact on the growth performance of Jian Carp (*Cyprinus carpio var. Jian*) and reported reduced growth rates at ≥60% replacement levels. In addition, Salem *et al.* [26] reported a significant reduction in growth, protein efficiency ratio, and enhanced feed conversion ratio in Nile tilapia (*Oreochromis niloticus*) when FM was replaced above 66.66% with silkworm pupae meal. Similarly, another widely used insect meal, black soldier fly (*Hermetia illucens*) larvae meal (BSFM) in aquaculture, known to improve the growth [18] and hepatic and intestinal histomorphology [17] of aquatic animals up to 60%, has also been shown to reduce the growth of aquatic animals above this level by causing a deficiency of essential amino acids [18]. This decreased growth performance following administration of diets containing high levels of insect meals has been suggested to be due to reduced diet digestibility and diet palatability [24, 27]. Ji *et al.* [25] also noted that the reduced growth performance of Jian carp by increasing SPM inclusion level could be due to the decreased feed intake because of reduced diet palatability. It is because the protein in the silkworm pupae meal is chitin-rich fibrion (5–10%), which has low digestibility in monogastric animals like fish [24, 27], causing poor protein digestion compared to soybean meal, which is full of polysaccharides and is easily digested in monogastric animals [28]. Therefore, the slightly reduced growth performance above 25% inclusion of SPM in the current study was presumably due to poor nutrient utilization with increasing SPM. Apart from promoting growth and feed utilization, the utilization of silkworm meal as a protein source is seeing growth within the aquaculture sector owing to its advantageous cost-effectiveness. In a study by Gurung *et al.* [29] evaluating the performance of locally formulated low-cost feeds for rainbow trout fry for 3 months, feed cost, total variable costs, and gross margin were significantly lower in treatment group SPM 100%, replacing the SM.

5. Conclusion

In conclusion, our study showed that SPM enhanced the growth performance of rainbow trout as body weight gain increased and feed conversion ratio decreased in response to dietary SPM. Therefore, to achieve the best results, SM and FFS replacement with 25% SPM is suggested, while it can be incorporated up to 37.5% without compromising growth performance.

6. References

1. Dheke S, Gbhaju SR. Growth response of rainbow trout (*Oncorhynchus mykiss*) on substitution of shrimp meal by different protein sources. Nepalese Journal of Zoology. 2013;1(1):24–29.
2. Choubert G, Luquet P. Utilization of shrimp meal for rainbow trout (Salmo gairdneri Rich.) pigmentation. Influence of fat content on the diet. Aquaculture. 1983;32(1–2):19–26.
3. Timalsina P, Yadav CNR, Lamsal GP, Acharya KP, Pandit NP. Effect of stocking density and source of animal protein on growth and survival of rainbow trout fingerlings in flow-through system at Nuwakot, Nepal. Aquac Rep. 2017;8:58–64.
4. Nepal AP, Basnet SR, Lamsal GP, Joshi PL, Mulmi RM. Economics of rainbow trout farming in Nepal. In: Cold water fisheries in the trans-Himalayan countries (eds. T. Petr and D.B. Swar). Rome. (FAO Fisheries Technical Paper., Rome); c2002.
5. Gurung TB, Basnet SR. Introduction of rainbow trout *Oncorhynchus mykiss* in Nepal: constraints and prospects. Aquaculture Asia. 2003;8(4):16–18.
6. Joshi PL, Lofvall LMW. Production technology and prospects of trout farming in Nepal. In: Swar DB, Pradhan GBN, Westland LMF, editors. National symposium on the role of fisheries and aquaculture in economic development of rural Nepal. Kathmandu: NEFIS; c1997. p. 27–34.
7. Voorhees TM. The potential to expand the rainbow trout (*Oncorhynchus mykiss*) commercial aquaculture industry in the mid-and-high-hills of Nepal. Ecological Aquaculture (ed Barry Costa-Pierce), 2011, 27.
8. Gurung TB, Wagle SK, Nepal AP, Lamsal GP. Rainbow trout (*Oncorhynchus mykiss*) based mountain aquaculture in Nepal. Nepalese Journal of Aquaculture and Fisheries. 1017;3(4):12–20.
9. Mohsen AA, Lovell RT. Partial substitution of soybean meal with animal protein sources in diets for channel catfish. Aquaculture. 1990;90(3–4):303–311.
10. Makkar HPS, Tran G, Heuzé V, Ankers P. State-of-the-art on use of insects as animal feed. Anim. Feed Sci. Technol. 2014;197:1–33.

11. Trivedy K, Kumar SN, Mondal M, Bhat CAK. Protein banding pattern and major amino acid component in de-oiled pupal powder of silkworm, *Bombyx mori* Linn. Journal of Entomology. 2007;5(1):10–16.
12. Rashmi KM, Chandrasekharaiah M, Soren NM, Prasad KS, David CG, Thirupathaiah Y, *et al*. Defatted silkworm pupae meal as an alternative protein source for cattle. Trop Anim Health Prod. 2022;54(5):327.
13. Sahu NK, Khajanji SN, Lakpale R. Effect of new pre-mix herbicides molecules on yield attributing character and yield of soybean [*Glycine max* (L.) Merrill] in *vertisols*. Int. J Adv. Chem. Res. 2022;4(2):199-202. DOI: 10.33545/26646781.2022.v4.i2c.101
14. Begum NN, Chakraborty SC, Zaher M, Abdul MM, Gupta M V. Replacement of fishmeal by low-cost animal protein as a quality fish feed ingredient for Indian major carp, *Labeo rohita*, fingerlings. J Sci Food Agric. 1994 Feb;64(2):191–197.
15. Rahimnejad S, Hu S, Song K, Wang L, Lu K, Wu R, *et al*. Replacement of fish meal with defatted silkworm (*Bombyx mori* L.) pupae meal in diets for Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture. 2019;510:150–159.
16. Bista JD, Wagle SK, Pradhan N, Roy NK. Nutrition and Pellet Feed Formulation for Rainbow Trout (*Oncorhynchus mykiss*) in Nepal. In: Gurung TB, editor. Scaling up of Rainbow trout farming strategies in Nepal. Godawari, Kathmandu: Fisheries Research Division; c2008.
17. Shakoori M, Gholipour H, Naseri S. Effect of replacing dietary fish meal with silkworm (*Bombyx mori*) pupae on haematological parameters of rainbow trout *Oncorhynchus mykiss*. Comp Clin Path. 2015;24(1):139–143.
18. Cummins VC, Rawles SD, Thompson KR, Velasquez A, Kobayashi Y, Hager J, *et al*. Evaluation of black soldier fly (*Hermetia illucens*) larvae meal as a partial or total replacement of marine fish meal in practical diets for Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture. 2017;473:337–344.
19. Melenchón F, de Mercado E, Pula HJ, Cardenete G, Barroso FG, Fabrikov D, *et al*. Fishmeal Dietary Replacement Up to 50%: A Comparative Study of Two Insect Meals for Rainbow Trout (*Oncorhynchus mykiss*). Animals. 2022 Jan 12;12(2):179.
20. Quang Tran H, Van Doan H, Stejskal V. Environmental consequences of using insect meal as an ingredient in aquafeeds: A systematic view. Rev Aquac. 2022 Jan 13;14(1):237–251.
21. Stadtlander T, Stamer A, Buser A, Wohlfahrt J, Leiber F, Sandrock C. *Hermetia illucens* meal as fish meal replacement for rainbow trout on farm. J Insects Food Feed. 2017 Sep;3(3):165–175.
22. Wang G, Peng K, Hu J, Yi C, Chen X, Wu H, *et al*. Evaluation of defatted black soldier flies (*Hermetia illucens* L.) larvae meal as an alternative protein ingredient for juvenile Japanese seabass (*Lateolabrax japonicus*) diets. Aquaculture. 2019;507:144–54.
23. Shakoori M, Gholipour H, Naseri S, Khara H. Growth, survival, and body composition of rainbow trout, *Oncorhynchus mykisss*, when dietary fish meal is replaced with silkworm (*Bombyx mori*) pupae. Archives of Polish Fisheries. 2016;24(1):53-57.
24. Longvah T, Mangthya K, Ramulu P. Nutrient composition and protein quality evaluation of eri silkworm (*Samia ricini*) prepupae and pupae. Food Chem. 2011;128(2):400–403.
25. Ji H, Zhang JL, Huang JQ, Cheng XF, Liu C. Effect of replacement of dietary fish meal with silkworm pupae meal on growth performance, body composition, intestinal protease activity and health status in juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquac Res. 2015; 46(5):1209-1221.
26. Salem MFL, Khalafalla MME, Saad IAI, EL-Hais AMA. Replacement of fish meal by silkworm, *Bombyx mori* pupae meal, in Nile tilapia, *Oreochromis niloticus* diets. Egyptian Society of Nutrition and Feeds. 2008;11(3):611–624.
27. Kroeckel S, Harjes AGE, Roth I, Katz H, Wuertz S, Susenbeth A, *et al*. When a turbot catches a fly: Evaluation of a pre-pupae meal of the Black Soldier Fly (*Hermetia illucens*) as fish meal substitute: Growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). Aquaculture. 2012;364-365:345–352.
28. Jintasataporn O, Chumkam S, Jintasataporn O. Substitution of Silkworm Pupae (*Bombyx mori*) for Fish Meal in Broodstock Diets for Snakeskin Gourami (*Trichogaster pectoralis*). J Agric Sci. Technol. A, 2011, 1(8).
29. Gurung GB, Rai S, Nepal AP, Gharti K. Performance of locally formulated low-cost feeds for rearing rainbow trout (*Oncorhynchus mykiss* Walbaun, 1792) fry in Nepal. Journal of Agriculture and Forestry University. 2019;3:173-179.
30. Jayaram M, Shetty H. Studies on the growth rates of catla, rohu and common carp fed on different formulated feeds. Mysore J Agric Sci. 1990;14(4):598–606.