Indian J. Fish., 70(1): 103-110, 2023
DOI: 10.21077/ijf.2023.70.1.121903-15

Effect of replacement of dietary fish meal with silkworm pupae meal and black soldier fly larvae meal as a combination diet on the growth and digestive performance of koi carp *Cyprinus carpio* var. *koi* in the nursery phase

JEYAPRAKASHSABARI SRIDHARAN[1], ANAND SAMRAJ[2], STEPHEN SAMPATH KUMAR JOHN[3] AND CHERYL ANTONY[4]
[1]*Fisheries College and Research Institute, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Thoothukudi - 628 008 Tamil Nadu, India*
[2]*Erode Bhavanisagar Centre for Sustainable Aquaculture (EBCeSA), Tamil Nadu Dr. J. Jayalalithaa Fisheries University Erode - 638 451, Tamil Nadu, India*
[3]*Directorate of Sustainable Aquaculture, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam - 611 002 Tamil Nadu, India*
[4]*Department of Aquaculture, Dr. M. G. R. Fisheries College and Research Institute, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Ponneri - 601 204, Tamil Nadu, India*
e-mail: anand@tnfu.ac.in

ABSTRACT

The feasibility of using silkworm pupae meal and black soldier fly larvae meal in combination to replace fish meal in koi carp fry was assessed. Two weeks-old koi fry (2.47±0.15 cm, 0.28±0.06 g) were used as experimental animals. Four iso-nitrogenous experimental diets (T1, T2, T3, T4) with 35±0.37% crude protein were formulated. In T1, T2 and T3, 50% of fish meal was replaced with silkworm pupae meal and black soldier fly larvae meal at different combinations, *i.e.,* T1 (25:75), T2 (50:50), T3 (75:25). In T4, 100% of fish meal was replaced with silkworm pupae meal and black soldier fly larvae meal at 50:50 ratio. Present research findings showed that T2 diet-fed fishes performed significantly better ($p<0.05$) than other diets in all the growth parameters *viz.,* mean length gain (3.13±0.01 cm), mean weight gain (3.13±0.02 g), percentage length gain (127±0.71%), percentage weight gain (1118±7.07%), specific growth rate (4.16±0.01%/day), feed conversion ratio (1.30±0.01), feed efficiency ratio (0.76±0.01), protein efficiency ratio (2.18±0.01) and survival rate (28±1.41%). Digestive enzyme activities were also higher in T2 diet-fed fishes. Therefore, insect meal can be effectively utilised to replace fish meal in the diet of koi fry.

Keywords: Black soldier fly larvae meal, Digestive enzymes, Growth performance, Koi carp fry, Silkworm pupae meal

Introduction

Globally, keeping ornamental fishes or 'living jewels' (Stratton, 1995) for decorative purposes owing to their attractive colour patterns has been one of the most popular hobbies since ancient times (Andrews, 1990). In 2014, 57% of the total global exports of ornamental fishes were contributed by Asian countries, with an export value of US$ 197.7 million. Besides that, Europe, South America, North America, Africa, Oceania and the Middle East are the other major ornamental fish exporters (Dey, 2016). Although enriched with vast biodiversity (Mahapatra, 2018), with an export value of 1.2 million US$ in 2017-18 (Gurvidhar, 2019), India contributed to only 1% of the total global ornamental fish trade (Satam *et al*., 2018), with more than 288 exotic species in the Indian ornamental fish trade market (Ghosh *et al*., 2003). In Indian domestic trade, koi carp (*Cyprinus carpio* var. *koi*), a colourful variant of common carp (Haniffa *et al*., 2007) and goldfish (*Carassius auratus*) alone account for nearly 45% of the total ornamental fish trade in the world (Jonathan, 2021).

The larval rearing phase in ornamental fish farming is more critical than other life stages. Protein must be supplied adequately for better survival, which is essential during the larval rearing phase of ornamental fishes. Moreover, the feeding success in the larvae-rearing stage depends upon the gradual development of physiological functions and the availability of suitable food items for the larvae (Ronnestad *et al*., 2013). Till now, fish meal has been used as the primary protein source in every aquafeed and the aquaculture sector alone consumed 68.2% of the total global fish meal produced (Tacon and Metian, 2008). The preference for fish meal in aquafeed is due to its high protein content, excellent amino acid profile, lack of anti-nutritional factors and better nutrient digestibility (Daniel, 2018). But due to the global stagnation in fish catches, the dietary inclusion of fish meal in aquafeed could decrease in the long term, coupled with increased costs (Tacon and Metian, 2008), which will be a hurdle for fish farmers, economically and technically (Rumsey, 1993). This increase in demand, coupled with rising costs, has put forth a search for an alternative protein source to replace fish meal (Hodar *et al*., 2020) in aquafeed. Insect meal as a significant protein source to substitute fish meal is affordable and anticipated in the future food security equation (Bazoche and Poret, 2021). Insect meals also contain high-quality protein and are an excellent lipid source with essential vitamins and minerals (DeFoliart *et al*., 2009), making them a better alternative for fish meal in aquafeed.

Among the various insects, the black soldier fly (*Hermetia illucens*) is remarkable for its capability to convert organic wastes into a high-quality protein source and for its application as a feedstuff for a wide variety of animals (Barragan-Fonseca *et al*., 2017). Black soldier fly larvae meal (BSF) is an emerging protein source with a crude protein content of 56.9% (Stamer *et al*., 2014) and has been used in fish feed since the 1970s (Bondari and Sheppard, 1981). The growth performance of juvenile Jian carp (*Cyprinus carpio* var. *jian*) was not affected by including this dietary black soldier fly larvae oil up to 100% (Li *et al*., 2016) in its diet. Reports from another study also show that BSF can replace 100% fish meal in the diet of Jian carp (Zhou *et al*., 2018) without negative impacts. Mamuad *et al*. (2021) found that common carp (*C. carpio*) fed with 30% soldier fly (*Ptecticus tenebrifer)* in the diet obtained maximum length gain. Amur carp (*C. carpio*) (Amala *et al*., 2018) and mirror carp (*Cyprinus carpio* var. *specularis*) (Xu *et al*., 2020) also showed better growth performance when fed with BSF.

Other than BSF, silkworm pupae (*Bombyx mori*) also have high nutritional value; particularly dried silkworm pupae meal (SWP) is a valuable source of protein (50-71%) and lipid (30%) (Meicai and Gaoqiang, 2001). Due to its high protein content, SWP can be used as feed for monogastric species such as poultry, piggery, and fish (Trivedy *et al*., 2008). Nandeesha *et al*. (1986) observed rohu (*Labeo rohita*) to perform better with a silkworm faecal-based diet rather than the fish meal and slaughterhouse waste-based diet. Catla-Rohu hybrid also showed higher final growth than the control feed when fed with 15% SWP in its diet (Nandeesha *et al*., 1988). Earlier research studies with catla (*Catla catla*) fingerlings also revealed that SWP can replace 100% fish meal with better growth and survival (Hasan, 1991). No negative impact on mirror carp fingerling (Ji *et al*., 2012) and Jian carp (Ji *et al*., 2015) were found when the fish meal was partially replaced with SWP in their diet. Moreover, the nutritional requirements of koi carp may vary according to age, size and environmental temperature. Ideally, they require crude protein content of 35% in their diet, with lipid levels below 15% (Lewbart, 1998) for better growth. Especially larval stage fishes require more protein in their diet than adult stage fishes. Hence, the present study focused on the effects of the highly proteinaceous insect larvae meals, such as BSF and SWP, in combination to replace 50% of the fish meal on the growth and digestive performance of koi carp fry in the nursery phase.

Materials and methods

Ethics statement

The present study evaluated the dietary inclusion of insect protein sources such as SWP and BSF in combination to replace fish meal in the diet of koi carp (*Cyprinus carpio* var. *koi*) fry. The present study adopted all the rules and regulations for experimental animal care and procedures stipulated by Tamil Nadu Dr J. Jayalalithaa Fisheries University (TNJFU), Nagapattinam, Tamil Nadu, India.

Experimental set up

The present study was conducted at Erode Bhavanisagar Centre for Sustainable Aquaculture (EBCeSA), Bhavanisagar, Erode, Tamil Nadu, India. The experimental set up consisted of four treatments (T1, T2, T3 and T4) and one control (C) in replicates (R1 and R2). The experimental happas of size 10x3x1 m were washed with soap oil and sun-dried before the installation in the earthen pond. Cleaned happas were installed in the earthen ponds using bamboo poles and covered with bird fencing nets as a bio-security measure. After installation, the happas were scrubbed fortnightly to eliminate algae and waste material to ensure proper water circulation and to prevent clogging.

Experimental animal

Two weeks old hatchery produced koi fry of size 2.47±0.15 cm and 0.28±0.06 g were transferred from the nursery pond and stocked into the experimental hapas at a stocking density of 3000 koi fry per hapa.

Experimental diets

Four iso-nitrogenous and iso-caloric experimental diets (T1, T2, T3 and T4) with 35±0.37% crude protein and 5±0.34% lipid were formulated. For the first three diets, T1, T2 and T3, 50% of fish meal was replaced with SWP and BSF as a combination diet at three different combinations *viz.,* T1-25:75, T2-50:50, T3-75:25. In the fourth diet T4, 100% of fish meal was replaced by SWP and BSF, each at 50%. These combinations of diets were made based on the formulation reported by Jahan *et al*. (2003). All the feed ingredients, such as fish meal, SWP, BSF, groundnut oil cake, rice bran, wheat flour and vitamin-mineral premix, were ground and mixed thoroughly in the pulveriser and feed homogeniser at the EBCeSA. The feed and proximate composition of all the experimental diets are depicted in Tables 1 and 2.

Assessment of proximate feed composition, such as crude protein and crude lipid content, was done at the Animal Feed Analytical and Quality Assurance Laboratory, TANUVAS, Namakkal, Tamil Nadu. Ash and moisture contents were analysed following standard protocols (AOAC, 2016) at EBCeSA. The gross energy values of the experimental diets were arrived at using the formula of Henken *et al*. (1986).

Feeding trial

During the experimental trial period of 60 days, mashed experimental feed at the rate of 5% of body weight was provided to the fish. Fortnightly sampling assessed the growth performance regarding length and weight gain. Post-sampling, fishes were released back into the respective hapas after a dip treatment in 0.1% potassium permanganate ($KMnO_4$) and in mild salt solution.

Water quality management

Water quality parameters *viz.,* Water temperature, pH, Dissolved oxygen, Ammonia-N, Nitrite-N, Nitrate-N, Inorganic phosphate, Free CO_2, Total hardness, Total alkalinity, Total suspended solids, Total dissolved solids, and Electrical conductivity, were analysed and recorded during every fortnightly sampling, following standard procedures (APHA, 2005). Duplicate water samples were taken from each treatment during every fortnightly sampling. The mean values of the water quality parameters recorded during the experimental trial are shown in Table 3.

Assessment of growth performance

Sampling was done fortnightly over 60 days to assess the growth performance of the fish. Based on the recorded data, the growth parameters such as mean length gain (MLG) (cm), mean weight gain (MWG) (g), percentage length gain (PLG) (%), percentage weight gain (PWG) (%), specific growth rate (SGR) (% per day), feed conversion ratio (FCR), the feed efficiency ratio (FER), protein efficiency ratio (PER) and survival rate (%), were calculated using the following formulae (Li *et al*., 2017):

MLG = Mean final length (cm) - Mean initial length (cm)

MWG = Mean final weight (g) - Mean initial weight (g)

$$PLG\ (\%) = \frac{\text{Final length (cm) - Initial length (cm)}}{\text{Initial length (cm)}} \times 100$$

$$PWG\ (\%) = \frac{\text{Final weight (cm) - Initial weight (g)}}{\text{Initial weight (g)}} \times 100$$

Table 2. Proximate composition of all the experimental diets

Proximate composition	C	T1	T2	T3	T4
Crude protein (%)	35.05	34.95	35.38	35.12	35.88
Crude lipid (%)	4.93	5.05	5.75	5.28	5.52
Moisture (%)	5.60	7.52	8.18	7.84	7.16
Ash (%)	13.59	11.22	10.60	10.52	8.76
Energy (MJ kg^{-1})	1.8393	1.8830	1.9119	1.9014	1.9422
Dry matter (%)	94.40	92.48	91.82	92.16	92.84

Table 3. Mean values of the water quality parameters recorded during the experiment trial

Parameters	Mean±SD
Temperature (◦C)	25.00±0.01
pH	8.30±0.01
Dissolved oxygen (ppm)	4.10±0.05
Total alkalinity (ppm)	31.20±0.03
Total hardness (ppm)	151.60±0.03
Free CO_2 (ppm)	7.20±0.01
Total dissolved solids (ppm)	0.76±0.05
Total suspended solids (ppm)	0.06±0.01
Ammonia (ppm)	0.32±0.03
Nitrite (ppm)	0.14±0.05
Nitrate (ppm)	2.64±0.02
Phosphate (ppm)	0.59±0.03
Electrical conductivity (µs cm^{-1})	0.36±0.04

Table 1. Feed composition of all the experimental diets

Feed ingredient	Experimental diets (g 100 g^{-1})				
	C	T1	T2	T3	T4
Fish meal[a]	29.40	14.70	14.70	14.70	-
Silkworm pupae meal[b]	-	3.68	7.35	11.02	14.70
Black soldier fly larvae meal[c]	-	11.02	7.35	3.68	14.70
Ground nut oil cake[d]	29.40	29.40	29.40	29.40	29.40
Rice bran[e]	24.70	24.70	24.70	24.70	24.70
Wheat flour[f]	16.40	16.40	16.40	16.40	16.40
Vitamin mineral pre-mix[g]	0.10	0.10	0.10	0.10	0.10

a-Pearl City Fish Meal Plant, Thoothukudi, Tamil Nadu; b-Silvermine Silk Processors Pvt. Ltd., Udumalpet, Tamil Nadu; c-Eco Care Agrovet, Pondicherry; d, e, f, g- Procured from local market around Bhavanisagar, Tamil Nadu; g-Ingredients included per kg: Vitamin A 700000 IU; Vitamin D_3 70000 IU; Vitamin E 250 mg; Cobalt 150 mg; Copper 1200 mg; Iodine 325 mg; Iron 1500 mg; Magnesium 6000 mg; Potassium 100 mg; Sodium 5.9 mg; Manganese 1500 mg; Sulphur 0.72%; Zinc 9600 mg; DL-Methionine 1000 mg; Calcium 25.5%; Phosphorus 12.75%

$$\text{SGR} = \frac{\text{In final weight (cm) - In initial weight}}{\text{Experimental duration in days}} \times 100$$

$$\text{FCR} = \frac{\text{Total dry feed fed (g)}}{\text{Total wet weight gain (g)}}$$

$$\text{FER} = \frac{1}{\text{FCR}}$$

$$\text{PER} = \frac{\text{Total wet weight gain (g)}}{\text{Dry weight of protein fed (g)}}$$

$$\text{Survival rate (\%)} = \frac{\text{Total no. of harvested animal}}{\text{Total no. stocked}} \times 100$$

Digestive enzyme activities

At the end of the 60-days feeding trial, random fish samples from each treatment were collected and stored in a deep freezer. Duplicate fish muscle samples were taken from each treatment at the end of the feeding trial. Tissue homogenate (5%) was prepared using a pestle and mortar and the homogenised samples were centrifuged at 5000 rpm for 10 min at 4°C. After centrifugation, 5 ml supernatant was collected in a test tube and stored at -20°C until further analysis. Standard procedures *viz.,* casein digestion method (Drapeau, 1976) for protease activity, Di-Nitro-Salicylic acid (DNS) method (Rick and Stagbauer, 1974) for amylase activity and titrimetric method (Cherry and Crandall, 1932) for lipase activity, were followed for the digestive enzyme analysis and results were expressed as µMmg protein^{-1}. As the fishes were too small to collect their digestive tract, all enzyme activities were analysed for fish muscle samples. Further, the metabolic aspartate aminotransferase (AST) and alanine aminotransferase (AST) activities in the fish muscle samples were determined by the method described by Wooten (1964) and the results were expressed as nM mg^{-1} min^{-1}.

Statistical analysis

All the collected data were processed and analysed by one-way ANOVA and Duncan's multiple range test using statistical software SPSS version 20.0 at 5% significance level to test for significant differences between the mean values of various treatments (SPSS Statistics for Windows, IBM. Version 20.0).

Results and discussion

The estimated growth performance of fish fed with different experimental diets is depicted in Table 4. After 60 days of the feeding trial, the highest mean final body length of 5.60±0.11 cm and mean final body weight of 3.41±0.12 g were obtained in T2 diet-fed fishes. Significantly higher ($p<0.05$) MLG of 3.13±0.01 cm and MWG of 3.13±0.02 g were recorded in T2 than in other treatments and control diet-fed fishes. Maximum PLG (127±0.71%), PWG (1118±7.07%) and significantly higher ($p<0.05$) SGR (4.16±0.01% day^{-1}) were also observed in the fishes fed with the T2 diet. Moreover, no significant difference ($p>0.05$) was observed between the treatments in terms of FCR, FER and PER, with control diet-fed fishes registering poor FCR (1.49±0.13), FER (0.67±0.06) and PER (1.91±0.16). The highest survival rate was recorded in T2 (28±1.41%) diet-fed fishes, with no significant difference ($p>0.05$) found between the treatments.

Digestive protease, amylase, lipase and metabolic AST, ALT enzyme activities of koi carp fry fed with different experimental diets are shown in Fig. 1 and 2.

Table 3. Growth performance recorded by the fishes fed with different experimental diets

Growth parameters	Diets				
	C	T1	T2	T3	T4
Mean initial length (cm)	2.47±0.15	2.47±0.15	2.47±0.15	2.47±0.15	2.47±0.15
Mean final length (cm)	4.48±0.15^a	5.26±0.14^b	5.60±0.11^c	5.20±0.14^b	5.12±0.25^b
Mean length gain (cm)	2.01±0.01^a	2.79±0.06^b	3.13±0.01^c	2.73±0.02^b	2.65±0.01^b
Percentage length gain (%)	81±0.71^a	113±2.83^b	127±0.71^c	111±0.71^b	107±0.71^b
Mean initial weight (g)	0.28±0.06	0.28±0.06	0.28±0.06	0.28±0.06	0.28±0.06
Mean final weight (g)	2.11±0.27^a	3.23±0.35^b	3.41±0.12^c	3.11±0.17^b	3.16±0.25^b
Mean weight gain (g)	1.83±0.16^a	2.95±0.13^b	3.13±0.02^c	2.83±0.01^b	2.88±0.05^b
Percentage weight gain (%)	654±55.86^a	1054±45.96^b	1118±7.07^c	1011±2.83^b	1028±17.68^b
Specific growth rate (% day^{-1})	3.36±0.13^a	4.07±0.07^b	4.16±0.0^c	4.01±0.01^d	4.03±0.03^b
Feed conversion ratio	1.49±0.13	1.24±0.06	1.3±0.01	1.31±0.01	1.28±0.02
Feed efficiency ratio	0.67±0.06	0.8±0.04	0.76±0.01	0.76±0.01	0.78±0.01
Protein efficiency ratio	1.91±0.16^a	2.29±0.1^b	2.18±0.01^b	2.17±0.01^b	2.22±0.04^b
Survival rate (%)	20±1.41^a	25±0.71^b	28±1.41^b	26±0.71^b	25±1.41^b

Values are presented as mean±standard deviation.
Values in the same row with different superscripts differ significantly ($p<0.05$) for each parameter.

Significantly highest ($p<0.05$) protease activity (15.66±0.30 µM mg protein^{-1}) and amylase activity (13.41±0.23 µM mg protein^{-1}) were recorded in T2 diet-fed fishes.

No significant differences ($p>0.05$) were observed between the treatments regarding lipase activity, with T2 diet-fed fish recording higher lipase activity of 22.47±0.16 µM mg protein^{-1}. The AST and ALT enzyme activities were also significantly higher ($p<0.05$) in T2 (12.82±0.23 nM mg protein^{-1} min^{-1}, 13.93±0.08 nM mg protein^{-1} min^{-1}), respectively compared to other treatments and control.

Results of the present study showed that T2, a combination of SWP and BSF at 50:50 ratio, replacing 50% fish meal, attained better growth performance than the other treatments, T1, T3, T4 and control. The T2 diet also performed 58 and 57% better than the control regarding MLG (cm) and PLG (%). The MWG (g) and PWG (%) were also 71% higher in T2 diet-fed fishes than in the control. In the present study, the SGR (% per day) was significantly higher ($p<0.05$) in T2 diet-fed fishes among all the treatments. At the same time, the estimated FCR and FER were not significantly different ($p>0.05$) among all the treatments and control. Also, the PER and survival rate (%) were not significantly different ($p>0.05$) among treatments, with the control diet-fed fishes attaining the least PER and survival rate (%). Similarly, 90-day feeding trial with rohu *(L. rohita)* fed on SWP at 50% replacement for fish meal resulted in better SGR (% day^{-1}), FCR and PER (Begum *et al.,* 1994). In a previous study with Amur carp, *C. carpio* fed with BSF up to 70% as a replacement for fish meal showed no significant differences ($p>0.05$) in the growth performance in terms of FCR, SGR (% day^{-1}) and PER (Amala *et al*., 2018). However, in the present study, all the experimental diets performed better than the control diet. Especially T4 diet, in which 100% fish meal was replaced with SWP and BSF, also performed better than the control, with no significant difference ($p>0.05$) in FCR (1.28±0.02), FER (0.78±0.01), PER (2.22±0.04) and survival rate (25±1.41%) among other treatments. Similarly, no significant difference ($p>0.05$) in the growth performance was found between the treatments replacing 100% fish meal with defatted BSF in the diet of Jian carp (Li *et al*., 2017). It has been reported that SWP can also replace upto 100% fish meal in the diet of common carp (*C. carpio*) (Nandeesha *et al*., 2000) with better growth and survival. However, concerning other treatments such as T1 and T3 in the present study, there was no significant difference ($p>0.05$) in the growth parameters *viz.,* MLG (cm), MWG (g), PLG (%), PWG (%), FCR, FER, PER and survival rate (%). Among T1 and T3, T1 performed slightly better than T3, possibly due to their higher amount of BSF as a protein source in the combination level.

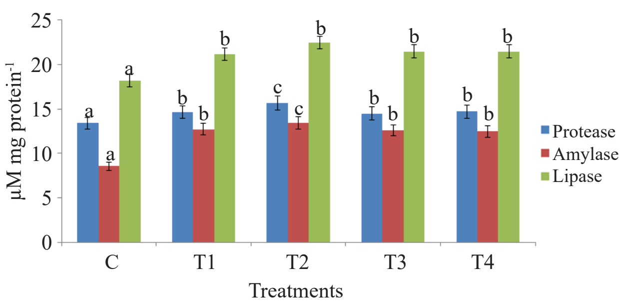

Fig. 1. Protease, amylase and lipase enzyme activity recorded in the experimental fishes. Bars with different superscripts differ significantly ($p<0.05$)

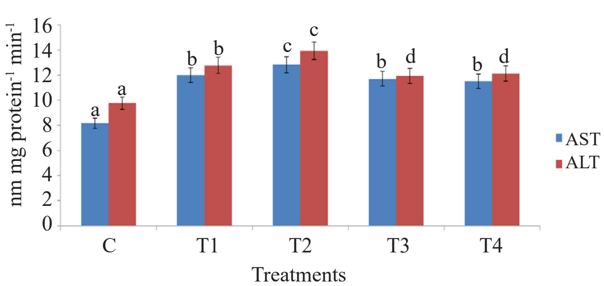

Fig. 2. AST and ALT enzyme activity recorded in the experimental fishes. Bars with different superscripts differ significantly ($p<0.05$)

In the present study, fishes fed the T2 diet had the highest protease, amylase, lipase, AST and ALT enzyme activities. However, this was not significantly different ($p>0.05$) from T1, T3 and T4. Similarly, Xu *et al*. (2020), in their research with mirror carp (*Cyprinus carpio* var. *specularis*) fed with black soldier fly larvae pulp, also reported no significant difference ($p>0.05$) among the treatments in digestive amylase and lipase activity. In the present study, all the treatment diet-fed fishes had increased protease, amylase and lipase enzyme activities than the control, which shows adequate protein and lipid utilisation in the respective diets. Improved protein and lipid utilisation are evident from the better growth performance in the treatments rather than control. Higher protease and lipase activities in fishes may be due to their attraction towards a protein-enriched diet rather than a carbohydrate-rich diet, as observed in an earlier study by Farhoudi *et al*. (2013) in common carp *(C. carpio)* larvae. Shcherbina *et al*. (1976) reported adaptive changes in the activity of the proteolytic enzymes in common carp concerning the type of diet. These previous research findings suggest that the higher enzyme activity levels in the present study indicate better feed utilisation and better growth achieved by the treatments rather than the control. Amino acids, the building blocks of proteins, alone contribute 14 to 85% of the energy requirements in teleost fishes (Ballantyne, 2001). A higher AST and ALT enzyme activity indicates effective metabolic utilisation of these amino acids (Jurss and Bastrop, 1995). Elevated AST activity in the muscle and hepatopancreas in Jian carp has been attributed to its role in amino acid catabolism (Jiang *et al*., 2015). Therefore, higher AST and ALT activities in the fishes of all treatments in the present study could be due to the effective utilisation of amino acid sources in the experimental diets.

In the present study, it was observed that fishes fed with SWP and BSF at different combination levels such as 25:75 (T1), 50:50 (T2), 75:25 (T3) ratio for 50% replacement of fish meal and SWP and BSF at 50:50 (T4) ratio as 100% replacement for fish meal showed better growth performance than the control diet fed fishes. Similarly, carps provided with a combination of alternative protein sources, such as poultry feather meal, meat meal and soy protein concentrate, each at 3% in the diet, showed no variation in the protein utilisation and protein efficiency ratio (Jahan *et al*., 2003). These observations from the present study reveal that SWP and BSF can be used as a combination diet to replace fish meal in the diet of koi carp fry for better growth and digestive performance in the nursery phase.

Based on the present research findings, it could be concluded that silkworm pupae meal and black soldier fly larvae meal can be effectively utilised as a substitute for fish meal. However, a replacement level of 50% fish meal with silkworm pupae meal and black soldier fly larvae meal, each at 50%, showed the best performance in terms of growth and digestive enzyme activities in koi carp fry in the nursery phase and this diet yielded 57% better growth in koi fry than the regular control diet.

Acknowledgements

The authors are thankful to the Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Tamil Nadu, India, for the necessary support and facilities extended for the research programme.

References

Amala, P. P., Khan, U. J. P. M. F., Ballal, B. D. C. R. and Hassan, M. A. 2018. Black soldier fly *(Hermetia illucens)* pre-pupae meal as a fish meal replacement in diet for Amur carp *(Cyprinus carpio)*. *J. Inland Fish. Soc. India*, 50(2): 33-38.

Andrews, C. 1990. The ornamental fish trade and fish conservation. *J. Fish Biol.,* 37: 53-59. https://doi.org/10.1111/j.1095-8649.1990.tb05020.x.

AOAC 2016. *Official methods of analysis,* 20th edn. In: George, W. L., Jr. (Eds.), *International Union of Pure and Applied Chemistry (IUPAC), and the Codex Alimentarius Commission.* AOAC International, Rockville, Maryland, USA.

APHA 2005. *Standard methods of water and wastewater,* 21st edn. American Public Health Association, Washington, DC, USA.

Ballantyne, J. S. 2001. Amino acid metabolism. *Fish Physiol.,* 20: 77-107. https://doi.org/10.1016/S1546-5098(01)20004-1.

Barragan-Fonseca, K. B., Dicke, M. and van Loon, J. J. 2017. Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed - A review. *Journal of Insects as Food and Feed*, 3(2): 105-120. https://doi.org/10.3920/JIFF2016.0055.

Bazoche, P. and Poret, S. 2021. Acceptability of insects in animal feed: A survey of French consumers. *Journal of Consumer Behaviour*, 20(2): 251-270. https://doi.org/10.1002/cb.1845.

Begum, N. N., Chakraborty, S. C., Zaher, M., Abdul, M. M. and Gupta, M. V. 1994. Replacement of fishmeal by low-cost animal protein as a quality fish feed ingredient for Indian major carp, *Labeo rohita*, fingerlings. *Journal of the Science of Food and Agriculture*, 64(2): 191-197. https://doi.org/10.1002/jsfa.2740640207.

Bondari, K. and Sheppard, D. C. 1981. Black soldier fly larvae as feed in commercial fish production. *Aquaculture*, 24: 103-109. https://doi.org/10.1016/0044-8486(81)90047-8.

Cherry, I. S. and Crandall Jr, L. A. 1932. The specificity of pancreatic lipase: Its appearance in the blood after pancreatic injury. *American Journal of Physiology-Legacy Content*, 100(2): 266-273. https://doi.org/10.1152/ajplegacy.1932.100.2.266.

Daniel, N. 2018. A review on replacing fish meal in aqua feeds using plant protein sources. *Int. J. Fish. Aquat. Stud.,* 6(2): 164-179.

DeFoliart, G., Dunkel, F. U. and Gracer, D. 2009. Toward a recipe file and manuals on "How to collect" edible wild insects in North America. *The Food Insects Newsletter*, 4(3): 1-9.

Dey, V. K. 2016. The global trade in ornamental fish. *Infofish International*, 4(16): 23-29.

Drapeau, G. R. 1976. Protease from *Staphyloccus aureus*. In: *Methods in enzymology*, 45: 469-475. https://doi.org/10.1016/S0076-6879(76)45041-3.

Farhoudi, A., Abedian, K. A., Nazari, R. M. and Makhdoomi, C. H. 2013. Changes of digestive enzymes activity in common carp (*Cyprinus carpio*) during larval ontogeny. *Iranian J. Fish. Sci.,* 12(2): 320-334.

Ghosh, A., Mahapatra, B. K. and Datta, N. C. 2003. Ornamental fish farming - Successful small scale aqua business in India. *Aquac. Asia*, 8(3): 14-16.

Gurvidhar, S. 2019. Pretty lucrative - India's surge in ornamental fish farming. *The Fish Site*. https://thefishsite.com/articles/pretty-lucrative-indias-surge-in-ornamental-fish-farming.

Haniffa, M. A., Benziger, P. A., Arockiaraj, A. J., Nagarajan, M. and Siby, P. 2007. Breeding behaviour and embryonic development of koi carp (*Cyprinus carpio*). *TAIWANIA-TAIPEI*, 52(1): 93.

Hasan, M. R. 1991. Studies on the use of poultry offal and silk worm pupae as dietary protein sources for Indian major carp, Catla (Hamilton) [Bangladesh]. *Part B: Livestock, Fisheries, Agricultural Engineering and Socio-economics*, 415-428.

Henken, A. M., Lucas, H., Tijssen, P. A. T. and Machiels, M. A. M. 1986. A comparison between methods used to determine the energy content of feed, fish and faeces samples. *Aquaculture*, 58(3-4): 195-201. https://doi.org/10.1016/0044-8486(86)90085-2.

Hodar, A. R., Vasava, R. J., Mahavadiya, D. R. and Joshi, N. H. 2020. Fish meal and fish oil replacement for aqua feed formulation by using alternative sources: A review. *J. Exp. Zool.,India*, 23(1): 13-21.

Jahan, P., Watanabe, T., Kiron, V. and Satoh, S. 2003. Balancing protein ingredients in carp feeds to limit discharge of phosphorus and nitrogen into water bodies. *Fish. Sci.,* 69(2): 226-233. https://doi.org/10.1046/j.1444-2906.2003.00612.x.

Ji, H., Cheng, X., Li, J., Zhang, J. and Liu, C. 2012. Effect of dietary replacement of fish meal protein with silkworm pupae on the growth performance, body composition, and health status of *Cyprinus carpio* var. *specularis* fingerlings. *J. Fish. China*, 36(10): 1599-1611.

Ji, H., Zhang, J. L., Huang, J. Q., Cheng, X. F. and Liu, C. 2015. 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.,* 46(5): 1209-1221. https://doi.org/10.1111/are.12276.

Jiang Jun, Lin Feng, Ling Tang, Yang Liu, Weidan Jiang and Xiaoqiu Zhou 2015. Growth rate, body composition, digestive enzymes and transaminase activities and plasma ammonia concentration of different weight Jian carp (*Cyprinus carpio* var. *Jian*). *Animal Nutr.,* 1(4): 373-377. https://doi.org/10.1016/j.aninu.2015.12.006.

Jonathan, P. 2021. *Ornamental fish industry data*. https://www.ofish.org/ornamental-fish-industry-data.

Jurss, K. and Bastrop, R. 1995. Amino acid metabolism in fish. In: Hochachka, P. W and Mommsen, T. P (Eds.), *Biochemistry and molecular biology of fishes.* Elseiver, Amsterdam, p. 159-189. https://doi.org/10.1016/S1873-0140(06)80010-X.

Lewbart, G. A. 1998. Clinical nutrition of ornamental fish. In: *Seminars in avian and exotic pet medicine*, 7(3): 154-158. https://doi.org/10.1016/S1055-937X(98)80007-8.

Li, S., Ji, H., Zhang, B., Tian, J., Zhou, J. and Yu, H. 2016. Influence of black soldier fly *(Hermetia illucens)* larvae oil on growth performance, body composition, tissue fatty acid composition and lipid deposition in juvenile Jian carp (*Cyprinus carpio* var. *jian*). *Aquaculture*, 465: 43-52. https://doi.org/10.1016/j.aquaculture.2016.08.020.

Li, S., Ji, H., Zhang, B., Zhou, J. and Yu, H. 2017. Defatted black soldier fly *(Hermetia illucens)* larvae meal in diets for juvenile Jian carp *(Cyprinus carpio* var. *jian)*: Growth performance, antioxidant enzyme activities, digestive enzyme activities, intestine and hepatopancreas histological structure. *Aquaculture*, 477: 62-70. https://doi.org/10.1016/j.aquaculture.2017.04.015.

Mahapatra, B. K. 2018. Ornamental fishery resources in India: Diversified option for livelihood improvement. In: Mahapatra, B. K. and Patra, S. B. (Eds.), *Proceedings of the National seminar on recent trends in fishery and ecological science,* 19 May 2018. Kolkata Cente of ICAR-Central Institute of Fisheries Education, Kolkata, West Bengal, India, p. 33-44.

Mamuad, L., Lee, S. H., Jeong, C. D., Ramos, S., Miguel, M., Son, A. R., Kim, S. H., Cho, Y. I. and Lee, S. S. 2021. Ornamental fish, *Cyprinus carpio*, fed with fishmeal replacement *Ptecticus tenebrifer* and *Tenebrio molitor*. *Aquac. Res.,* 52(3): 980-990. https://doi.org/10.1111/are.14953.

Meicai, W. and Gaoqiang, L. 2001. The research and exploitation of insect protein. *Journal of Central-south Forestry College*, 21(2): 86-90.

Nandeesha, M. C., Devaraj, K. V. and Sudhakara, N. S. 1986. Growth response of four species of carps to different protein sources in pelleted feeds. *Proceedings of the First Asian Fisheries Forum, Asian Fisheries Society,* Manila, Philippines, p. 26-31.

Nandeesha, M. C., Srikanth, G. K., Varghese, T. G., Keshavanath, P. and Shethy, H. P. C. 1988. Influence of silkworm pupae based diets on grown organoleptic quality and biochemical composition of catla-rohu hybrid. In: *Aquaculture research in Asia. Management techniques and nutrition. Proceedings of the Asian Seminar on Aquaculture.* International Foundation for Science, Malang, Indonesia, p. 211-220.

Nandeesha, M. C., Gangadhara, B., Varghese, T. J. and Keshavanath, P. 2000. Growth response and flesh quality of common carp, *Cyprinus carpio* fed with high levels of non defatted silkworm pupae. *Asian Fish. Sci.,* 13(3): 235-242.

Rick, W. and Stagbauer, H. P. 1974. Amylase measurement of reducing groups. In: Bergmeyer, H. V. (Ed.), *Methods of enzymatic analysis*. Academic Press, New York, USA, p. 885-889. https://doi.org/10.1016/B978-0-12-091302-2.50074-8.

Ronnestad, I., Yufera, M., Ueberschar, B., Ribeiro, L., Saele, O. and Boglione, C. 2013. Feeding behaviour and digestive physiology in larval fish: Current knowledge, and gaps and bottlenecks in research. *Rev. Aquac.,* 5(1): S59-S98. https://doi.org/10.1111/raq.12010.

Rumsey, G. L. 1993. Fish meal and alternate sources of protein in fish feeds update. *Fisheries*, 18(7): 14-19. https://doi.org/10.1577/1548-8446(1993)018%3C0014:FMAASO%3E2.0.CO;2.

Satam, S. B., Sawant, N. H., Ghughuskar, M. M., Sahastrabuddhe, V. D., Naik, V. V., Pagarkar, A. U., Chogale, N. D., Metar, S. Y., Shinde, K. M., Sadawarte, V. R. and Sawant, A. N.

2018. Ornamental fisheries: A new avenue to supplement farm income. *Advanced Agricultural Research and Technology Journal*, 2(2): 193-197.

Shcherbina, M. A., Trofimova, L. N. and Kazlaskene, O. P. 1976. The activity of protease and the intensity of protein absorption with the introduction of different quantities of fat into the food of the carp *Cyprinus carpio*. *J. Ichthyol.,*16: 632-636.

Stamer Andreas, Wessels, S., Neidigk Ralph and Hoerstgen-Schwark, G. 2014. Black soldier fly (*Hermetia illucens*) larvae-meal as an example for a new feed ingredients' class in aquaculture diets. *Proceedings of the 4th ISOFAR Scientific Conference*. 'Building Organic Bridges', at the Organic World Congress. Istanbul, Turkey, p. 1043-1046.

Stratton, R. L. 1995. Some suggestions for the marine community tank. *Tropical fish hobbyist.* December, p. 62- 72.

Tacon, A. G. and Metian, M. 2008. Global overview on the use of fish meal and fish oil in industrially compounded aqua feeds. Trends and future prospects. *Aquaculture*, 285(1-4): 146-158. https://doi.org/10.1016/j.aquaculture.2008.08.015.

Trivedy, K., Kumar, S. N., Mondal, M. and Kumar Bhat, C. A. 2008. Protein banding pattern and major amino acid component in de-oiled pupal powder of silkworm, *Bombyx mori* Linn. *J. Entomol.,*5(1): 10-16.

Wooten, I. D. P. 1964. Microanalysis. In: Churchill, A. (Ed.), *Medical Biochemistry,* London, UK, p. 101-107.

Xu, X., Ji, H., Yu, H. and Zhou, J. 2020. Influence of dietary black soldier fly *(Hermetia illucens Linnaeus)* pulp on growth performance, antioxidant capacity and intestinal health of juvenile mirror carp *(Cyprinus carpio* var. *specularis)*. *Aquac. Nutrit.,* 26(2): 432-443. https://doi.org/10.1111/anu.13005.

Zhou, J. S., Liu, S. S., Ji, H. and Yu, H. B. 2018. Effect of replacing dietary fish meal with black soldier fly larvae meal on growth and fatty acid composition of Jian carp *(Cyprinus carpio* var. *jian)*. *Aquac.Nutrit.,* 24(1): 424-433. https://doi.org/10.1111/anu.12574.

Date of Receipt : 02.03.2022

Date of Acceptance : 16.01.2023