



Utilization of Black Soldier Fly Larvae as a Substitute for Fishmeal in Koi Carp (*Cyprinus carpio*) Diets: Effects on Growth, Digestive Enzyme Activity and Color Intensity

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ABSTRACT

Black soldier fly larvae (*Hermetia illucens*) represent a viable, sustainable protein source for aquaculture. However, its application in ornamental species, such as koi carp, which are economically vital, requires species-specific responses to maintain market value. This study aimed to investigate the growth performance, digestive enzyme activity, and color intensity of koi carp (*Cyprinus carpio*) fed black soldier fly larvae at 0, 25, 50, and 75% of the diet instead of fish meal. The carp were conducted with a treatment diet in glass tanks for 60 days. The research concluded that black soldier fly larvae can substitute fishmeal in carp diets. However, koi carp fed a meal supplemented with over 50% black soldier fly larvae had significantly lower final body weight, average weight increase, and average daily growth rate ($P<0.05$) compared to the control group and those fed a diet with a 25% replacement diet. The fish that consumed a diet with black soldier fly larvae instead of 75% had lower protease enzyme activity than the control group ($P<0.05$). In comparison, fish groups fed diets with black soldier fly larvae as a replacement for 50 and 75% showed considerably higher amylase and lipase activity than the control group ($P<0.05$). The L^* , a^* , and b^* values for skin color intensity above the lateral line were not significantly different across groups ($P>0.05$). Therefore, black soldier fly larvae can replace fishmeal in carp diets not exceeding 25% without affecting growth, variations in digestive enzymes, and skin color. This information could improve koi carp culture and promote aquaculture to satisfy future sustainable demand.

Keywords: Alternative protein, Aquatic animal feed, Growth, Digestive enzyme, Skin color.

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INTRODUCTION

The ornamental aquaculture industry has been continuously expanding, driven by the demand for pet fish. Advances in aquaculture have significantly improved the efficiency of production and the economic value of ornamental fish. The koi carp (*Cyprinus carpio* var. koi) market is economically important due to its worldwide popularity, especially in Asia and Europe, where demand for high-quality koi carp has driven continuous development in aquaculture (FAO, 2020). Diet plays an

important role in promoting growth, color development, and efficiency of various body systems. (NRC, 2011). Moreover, carotenoid supplementation in fish diets is essential for preserving color quality, which is chiefly responsible for the vibrant hues found in ornamental fish. Coloration substantially impacts the perceived value and consumer preference of ornamental fish among aquarists (Chatzifotis et al., 2005; Elbahnaawy & Elshopakey, 2024; Tran et al., 2025). Therefore, a diet that promotes both development and pigmentation has been the main focus in ornamental aquaculture research (Linh et al., 2024).

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Diets are frequently designed with high protein content, utilizing fishmeal as the primary protein source. The significant reliance on fishmeal raises issues about sustainability, price fluctuations, and environmental impacts (Zulkifli et al., 2022). Insect protein is one of the promising options and is attracting growing interest as a sustainable alternative to reduce the use of conventional fishmeal in aquaculture (Van Huis, 2013; Tippayadara et al., 2021; Gadzama, 2025; Zambrano Vera et al., 2025). The black soldier fly, *Hermetia illucens* (Linnaeus, 1758), belongs to the family Stratiomyidae. Insects serve as an alternative protein source that has garnered significant interest owing to their substantial nutritional value, particularly containing up to 8-60% protein and 18-40% fat (Barragán-Fonseca et al., 2017; Gadzama, 2025; Su et al., 2025). In addition, black soldier fly larvae contain carotenoids at 2.15mg/kg, which is important for promoting fish color (Secci et al., 2018). They can be grown from agricultural and organic waste and serve as an important tool for efficiently converting waste into protein-rich biomass, making them an essential resource for the circular bioeconomy (Anokye, 2025). This benefit has led to ongoing research on the utilization of black soldier fly larvae to develop animal feed (da Silva & Hesselberg, 2020; Salahuddin et al., 2024; Karhale et al., 2025). Considering the growing need for animal protein, expected to double by 2030, black soldier fly larvae present a viable, sustainable alternative protein source. Further research is required to elucidate the effects of black soldier fly larvae supplementation on animal responses as a substitute for conventional animal feed (Gadzama, 2025).

In the field of aquatic animal feed, a study investigates the use of black soldier fly larvae as an alternative to fish meal in formulations. It can promote the growth of various fish species, including the fighting fish (*Betta splendens*) (Kari et al., 2023), the rainbow trout (*Oncorhynchus mykiss* Walbaum) (Renna et al., 2017; Zhao et al., 2023) and the Japanese eel (*Anguilla japonica*). However, the effects may vary depending on the volume of replacement and species-specific needs, as the dietary inclusion of black soldier fly larvae has been associated with variations in digestive enzyme activity, which can affect feed efficiency and nutrient assimilation (Kuo et al., 2022).

For ornamental fish such as koi, pigmentation is a vital and fundamental attribute influencing marketability. Pigmentation is primarily affected by the consumption of carotenoids and natural pigments that accumulate in the skin and muscle tissues. Black soldier fly larvae naturally contain lipids that enhance pigment absorption (Tu et al., 2022).

Despite research on the use of black soldier fly larvae meal as a substitute for fishmeal in various economically important fish species, knowledge of its application in fancy carp (*Cyprinus carpio*), an ornamental fish with distinct nutritional requirements and significant value, remains limited. Moreover, studies on other fish species have indicated disparate effects of black soldier fly larvae, rendering it infeasible to derive definitive findings on its practical application. This study aims to evaluate the comprehensive impacts of black soldier fly larvae on growth performance, digestive enzyme activity, and skin

color intensity in fancy carp, to find the optimal replacement level for use in carp feed, thereby promote sustainable and efficient carp culture development.

MATERIALS & METHODS

Black Soldier Fly Larvae and Diet Preparation

The larvae of the black soldier fly larvae from the black soldier fly larvae farm in Samut Sakhon Province of Thailand were dried in a hot air oven at 60°C for 48 hours. They were minced using a grinder into 1-2mm for use in the feed formulas. The other feedstuffs in Table 1 were ground through a 1-2mm sieve, weighed, and mixed by a mixer machine. They were taken to an extruder and then processed the feedstuffs into pellets with a diameter of 2-3mm. The diets were dried for 24 hours at room temperature and stored in sealed plastic bags at -20°C before use. The experimental diets were proximated for chemical composition, including protein, fat, fiber, ash, and moisture, according to AOAC (2000). Nitrogen-free extract (NFE) was calculated (100 - (protein + fat + ash + fiber)), and total carotenoids were analyzed following Howe & Tanumihardjo (2006). Ingredients, proximate composition and total carotenoid of experimental diets are summarized in Table 1.

Table 1: Ingredients, proximate composition and total carotenoid of experimental diets

Ingredients (g kg ⁻¹)	BSFL 0	BSFL 25	BSFL 50	BSFL 75
Fish meal	250	187.5	125	62.5
Black soldier fly meal	0	62.5	125	187.5
Shrimp head meal	50	50	50	50
Squid liver meal	60	60	60	60
Soybean meal	150	150	150	150
Broken rice meal	140	140	140	140
Rice bran	50	50	50	50
Yeast	50	50	50	50
Wheat flour	120	120	120	120
Premix ¹	10	10	10	10
Vitamin ²	10	10	10	10
Squid liver oil	20	20	20	20
Vegetable oil	20	20	20	20
Spirulina	50	50	50	50
Lecithin	10	10	10	10
Binders	10	10	10	10
Composition (%)				
Crude protein	33.65	33.58	31.35	30.56
Crude fat	7.33	11.18	12.05	13.98
Crude fiber	3.17	2.63	3.70	3.67
Ash	8.97	8.15	7.29	6.59
Moisture	7.20	7.07	6.63	6.36
NFE	39.68	37.99	38.98	38.84
Total carotenoid (μg/g)	0.39	1.20	1.27	1.41

¹Premix; ²Vitamin supplemented contains: (IU kg⁻¹ or g kg⁻¹ diet): Vitamin A 6,000,000IU, Vitamin D3 1,200,000IU, Vitamin E 6,000IU, Vitamin K3 0.45g, Vitamin B1 0.45g, Vitamin B2 3.5g, Vitamin B12 0.006g, Pantothenic Acid 5.4g, Nicotinic Acid 13.5g, Choline Chloride 85g, Copper 56g, Manganese 21g, Iron 29.6g, Zinc 33.5g, Iodine 0.3g, DL-Methionine 24.5g, L-Lysine 49g.

Fish and Experimental Procedures

Two hundred koi carp fingerlings were acquired from a commercial farm in Samut Sakhon Province of Thailand. The animals were accumulated for 7 days in cement ponds (1 cm³), which were constantly aerated and fed commercial ornamental fish pellets twice a day at 7:30 AM and 5:30 PM before the experiment. Twelve fish were sampled and set in each glass tank measuring 80x50x50cm, for a total of 12 tanks. Each tank was continuously filtered and aerated

throughout the trial. All fish were fed the treatment diet twice daily at 7:30 AM and 5:30 PM to apparent satiation, with the volume of feed recorded over 60 days. Additionally, throughout the trial, the tank was cleaned, and the water was changed every 7 days.

Growth Performance

Data were obtained by selecting at random eight fish from each tank to record their length, weight, and number of fish surviving at the beginning and throughout the trial. The growth performance has been evaluated for the average final body weight (FBW), average weight gain (AWG), average daily growth rate (ADG), feed conversion ratio (FCR) and survival rate (SR), following De Silva & Anderson (1994).

Digestive Enzyme Analysis

Three fish were randomly selected from each tank. The abdominal cavity of the fish was cut open using an aseptic procedure to obtain organ samples, including the intestines and liver. The organ sample was prepared for crude enzyme extraction by homogenizing it in Tris-HCl buffer (50 mM, pH 7.5) in a tube. The homogenate was centrifuged at 15,000g for 5min at 4°C. The supernatant has been stored in a vial at -20°C for enzyme analysis (Gimenez et al., 1999). Protease analysis was conducted in triplicate by quantifying the increase in cleavage of short-chain polypeptides, with minor modifications relative to Bezerra et al. (2005). The total protease activity was assessed using a 1% (w/v) azocasein solution prepared in buffers at different pH values (6-8). The substrate (500µL) was incubated with crude extract (20µL) and buffer solution (200mL) for 60min at 30°C. Subsequently, add 500µL of 20% (w/v) trichloroacetic acid (TCA), then centrifuge at 15,000g at 4°C for 10min. One milliliter of supernatant was combined with 1 M NaOH (1.5mL), and the absorbance was measured at 440nm using a spectrophotometer. The protease-specific activity was expressed by the change in absorbance per minute per mg of protein of the enzyme extract ($\Delta\text{Abs min}^{-1} \text{ mg protein}^{-1}$). The amylase enzyme was analyzed in triplicate utilizing the 3,5-dinitrosalicylic acid (DNS) method, as modified by Bernfeld (1951). A starch substrate (1% w/v) was formulated in buffers at various pH levels (6-8). The substrate (500µL) was incubated with crude extract (20µL) and buffer solution (400µL) for 60min at 30°C. Subsequently, 1.5mL of 1% dinitrosalicylic acid (DNS) solution was added and boiled for 5min. Subsequently, 1.5mL of distilled water was added to the combination and the cooled solution. The cooled solution was analyzed at 550nm utilizing a spectrophotometer. Maltose (10-100mM) was used to construct the calibration curve. The amylase-specific activity was quantified as the mmol of maltose produced per min per mg of protein. Analyses of lipase enzyme were conducted in triplicate with the modified method of Markweg-Hanke et al. (1995), 0.01M para-nitrophenylpalmitate (pNNP) dissolved in iso-isopropanol as the substrate. The substrate (100µL) was incubated with crude enzyme extract (50µL) and buffer solution (800µL) for 60 min at 30°C. Subsequently, 250µL

of 0.1M Na₂CO₃ was added, and the mixture was centrifuged at 10,000g at 4°C for 15min. Absorbance was measured at 410nm using a spectrophotometer. Para-nitrophenol (pNP) was employed in a concentration of 100–1000µg/mL for the calibration curve. Lipase-specific activity was evaluated by measuring the µmol of p-nitrophenol generated per min per mg protein.

Color Measurement

The six fish were randomly selected per tank at the beginning and every 15 days thereafter until the end of the experiment to be rendered unconscious using the clove oil for color measurement. The skin above the lateral line on the left side of the koi carp was analyzed using HunterLab MiniScan EZ 45/0 (LAV) within the CIE L*a*b* color space. The obtained L*, a*, and b* values were used for presenting lightness, redness or greenness, and blueness or yellowness, respectively (Hunter and Harold, 1987)

Water Quality

Water quality in the experimental tank was measured, including water temperature, dissolved oxygen, and pH, using a Hach multivariate water quality meter (HQ40D). Total ammonia and nitrite content were measured using the titration method (APHA, 1992).

Statistical Analysis

The data in the experiment was used to determine the difference of one-way analysis in variance (ANOVA). Duncan's New Multiple Range Test (DMRT) at the 95% confidence level ($P<0.05$) was used to determine differences among treatment means. Statistical analyses were performed using SPSS Statistics software.

RESULTS

Growth Performance

Koi carp fed with experimental diets showed different growth performance, as shown in Table 2. The fish fed diets with fish meal replaced with black soldier fly larvae at 0 and 25% exhibited equivalent average final weights ($P>0.05$), which were significantly greater than those in the groups fed diets with 50% and 75% substitution ($P<0.05$). The average weight gain and average daily growth rate decreased in fish fed diets replacing over 50% of the fish meal with black soldier fly larvae. The control group has the lowest feed conversion ratio ($P<0.05$) among the other treatment groups. The fish group that was fed a diet replacing 75% of fish meal with black soldier fly larvae exhibited the highest feed conversion ratio values ($P<0.05$). The survival rate across all treatment groups ranged from 97.22 to 100% and showed no statistically significant differences ($P>0.05$).

Digestive Enzyme

The protease enzyme activity in koi carp tended to decrease in the group that was fed a diet using black soldier fly larvae as a replacement for fish meal in treatment diets. The control group of fish had significantly higher activity values than the group fed with 75% of black

soldier fly larvae instead of fish meal ($P<0.05$). However, amylase activity tended to increase with the replacement level. The group fed with black soldier fly larvae replacing fish meal at 75% had the highest activity value and was statistically significantly different ($P<0.05$) from the other groups. In addition, lipase activity also increased. The control group and the group fed black soldier fly larvae replacing fish meal at 25% ($P>0.05$) had lower values than the groups fed 50% and 75% substitution diets ($P<0.05$). Both groups had statistically significant differences ($P<0.05$), as shown in Table 3.

Table 2: Growth performance of koi carp (*Cyprinus Carpio*) fed treatment diet for 60 days

Growth rate	BSFL 0	BSFL 25	BSFL 50	BSFL 75
IBW (g)	12.77±0.53 ^a	12.76±0.03 ^a	12.77±0.59 ^a	12.73±0.17 ^a
FBW (g)	28.49±0.18 ^c	28.14±0.34 ^c	27.01±0.01 ^b	26.54±0.07 ^a
AWG (g)	15.72±0.70 ^b	15.38±0.32 ^b	14.24±0.59 ^a	13.81±0.14 ^a
ADG (g/day)	0.35±0.02 ^b	0.34±0.01 ^b	0.32±0.01 ^a	0.31±0.01 ^a
FCR	2.65±0.09 ^a	2.90±0.04 ^b	3.27±0.07 ^c	3.28±0.05 ^d
SR (%)	100±0.00 ^a	97.22±4.81 ^a	100±0.00 ^a	100±0.00 ^a

Values (mean±SD) within a row with the same superscript are significantly different ($P<0.05$); IBW: Initial Body Weight; FBW: Final Body Weight; AWG: Average Weight Gain ADG: Average Daily Growth Rate; FRC: Feed Conversion Ratio; SR: Survival Rate.

Table 3: Digestive enzyme of koi carp (*Cyprinus Carpio*) fed treatment diet for 60 days. (Mean±SD)

Enzyme	BSFL 0	BSFL 25	BSFL 50	BSFL 75
Protease (U/mg protein)	2.87±0.27 ^b	2.20±0.43 ^{ab}	2.22±0.32 ^{ab}	1.89±0.35 ^a
Amylase (U/mg protein)	2.54±0.33 ^a	2.00±0.54 ^a	5.41±0.55 ^b	8.47±1.37 ^c
Lipase (U/g)	27.22±0.81 ^a	33.85±1.45 ^b	35.41±1.52 ^b	38.33±0.9 ^c

Values (mean±SD) within a row with common superscript are significantly different ($P<0.05$).

Color Measurement

The color value of the skin above the lateral line of fancy carp is shown in Table 4. Initially, the L*, a* and b* had no significant difference between all treatment groups ($P>0.05$). However, the L* and b* value tended to decrease in all groups for 15 days but there was no significant difference ($P>0.05$). The color of a* value shown an increase remain constant until the end of the experiment ($P>0.05$). However, the L*, a*, and b* values exhibited no significant differences among groups throughout the trial ($P>0.05$).

Table 4: Color intensity of koi carp (*Cyprinus Carpio*) fed treatment diet for 60 days

Parameter	Periods	BSFL 0	BSFL 25	BSFL 50	BSFL 75
L*	Initial	57.41±4.13 ^a	60.43±3.26 ^a	60.26±3.97 ^a	59.16±3.73 ^a
	15th day	54.44±0.68 ^a	55.56±3.17 ^a	52.75±0.64 ^a	53.61±0.96 ^a
	30th day	49.76±1.62 ^a	49.07±2.72 ^a	48.79±0.51 ^a	49.72±1.83 ^a
	45th day	51.69±0.88 ^a	51.70±0.52 ^a	51.13±0.41 ^a	51.61±0.10 ^a
	60th day	55.24±1.23 ^a	54.47±0.35 ^a	55.01±0.69 ^a	54.98±0.43 ^a
a*	Initial	3.20±0.89 ^a	2.91±0.16 ^a	3.00±0.06 ^a	3.16±0.36 ^a
	15th day	7.52±0.69 ^a	7.16±0.28 ^a	7.58±1.07 ^a	7.28±0.50 ^a
	30th day	7.02±0.53 ^a	7.23±0.49 ^a	7.34±0.53 ^a	7.12±0.84 ^a
	45th day	7.48±0.40 ^a	7.29±0.14 ^a	7.48±0.24 ^a	7.33±0.28 ^a
	60th day	8.34±0.22 ^a	8.19±0.62 ^a	8.86±0.62 ^a	8.73±0.53 ^a
b*	Initial	31.66±1.84 ^a	31.20±0.37 ^a	31.54±1.17 ^a	31.52±4.63 ^a
	15th day	25.87±0.40 ^a	25.21±1.69 ^a	25.22±1.21 ^a	25.23±0.93 ^a
	30th day	22.97±0.72 ^a	22.63±0.76 ^a	22.45±0.47 ^a	23.32±0.31 ^a
	45th day	21.65±0.44 ^a	21.33±0.73 ^a	21.29±0.43 ^a	21.31±0.69 ^a
	60th day	21.06±0.38 ^a	21.08±0.24 ^a	21.32±0.54 ^a	21.40±0.17 ^a

Values (mean±SD) within a row with common superscript are significantly different ($P<0.05$).

Water Quality

Throughout the experimental period, water quality in

the tank showed a temperature range from 28.07 to 32.75°C, a dissolved oxygen level of 6.29-7.41 ppm, a pH of 7.61-8.20, total ammonia concentrations of 0.06-0.22 ppm, and nitrite levels ranging from 0.00 to 0.078 ppm.

DISCUSSION

The growth rate of koi carp diminished in accordance with the proportion of black fly soldier larvae substituted in the experimental diet. Carp fed a diet with fish meal substituted by black fly larvae at 0% and 25% demonstrated appropriate growth, with no significant differences observed ($P>0.05$). However, carp receiving a diet with 50% and 75% replacement of black soldier fly larvae demonstrated a marked decline in growth performance ($P<0.05$). The observed growth revealed that koi carp exhibit a reduced growth response when fish food is substituted with a higher proportion of black army fly larvae. The larvae of the black soldier fly possess a substantial fat content ranging from 7% to 39% (Barragán-Fonseca et al., 2017). The elevated fat content in food can adversely affect nutritional intake, leading to imbalances in specific essential amino acids in insects (St-Hilaire et al., 2007; Li et al., 2016). This study found fat content from 11.18 to 13.98% in the treatment diet, which (more than 60 g/kg) would inhibit weight gain and increase lipid degradation in the hepatopancreas, thus affecting growth performance and feed utilization (Fan et al., 2021). The fish ingest large quantities, adversely impacting the efficiency of protein use for development performance (Makkar et al., 2014). This is consistent with the study of Du et al. (2006), which found that increasing fat level in diets fed to *Ctenopharyngodon idella* negatively affected the growth rate by decreasing food intake, reducing growth, lowering feed efficiency, and increasing visceral adipose tissue weight. Additionally, growth performance was reduced, which may be associated with chitin, a component of insects (black soldier fly larvae). Chitin is classified as an antinutritional factor, which limits the utilization of feed during digestion (Rust, 2003). Its presence has a detrimental effect on digestive efficiency, reducing nutrient absorption (Barroso et al., 2014; Henry et al., 2015; Marono et al., 2015; Guerreiro et al., 2021). Moreover, the study similar with Kroeckel et al. (2012) found that the replacement of fish meal with dried black soldier fly larvae in the diets of flounder (*Psetta maxima*) at levels of 0, 17, 33, 49, 64, and 76% resulted in a decline in growth rate as the inclusion of black soldier fly larvae increased, attributable to the elevated chitin content in the diet. Consequently, fish in the treatment groups required more feed to convert nutrients into growth, as indicated by the feed conversion ratio.

However, research has shown varying results of black fly soldier larvae usage in fish diet. Khieokhajonkhet et al. (2022) found that using black soldier fly larvae to replace fish meal in goldfish (*Carassius auratus*) diets at 0, 10, 20, 40, 60, 80, and 100% led to an increase in growth rate that was correlated to the amount of black soldier fly larvae in the diet. Tippayadara et al. (2021) investigated diets substituting fish meal with black soldier fly larvae

meal at levels of 0, 10, 20, 40, 60, 80, and 100%, and found no statistically significant differences in growth rates. The variation in growth performance outcomes is associated with species, fish size, insect types, insect consumption, and insect processing (Tscherirner & Simon, 2015; Lu et al., 2020).

The survival rates in this study were more than 97% and did not significantly differ ($P>0.05$). Das (2023) studied the use of insects, including grasshoppers, crickets, mealworms, and black soldier flies, to raise swordtail larvae (*Xiphophorus helleri*) compared with a commercial diet. The survival rate of swordtails fed on black soldier fly larvae was statistically different from that of those maintained on commercial artificial feed ($P>0.05$), indicating that the replacement of black soldier fly larvae did not harm fish survival.

Furthermore, the activity of digestive enzymes in koi carp varied in response to their digestive system's adaptation to the changing nutritional composition of their diet. Protease activity tended to decrease as the black soldier fly larvae substitution level increased. It was lowest in the fish group that fed a diet substituting 75% of fish meal with black soldier fly larvae ($P<0.05$) and was highest in the control diet. The reduction in protease activity was associated with a lower percentage of protein in treatment diets that incorporated a higher proportion of black soldier fly larvae as a substitute for fish meal, resulting in a comparable decline in protease activity essential for protein digestion. The chitin concentration in the composition of black soldier fly larvae influences the digestion and use of protein nutrients (Alegbeleye et al., 2012; Li et al., 2016). The study of Kamalii et al. (2022) found that the replacement of fish meal with black soldier fly larvae in the diets of goldfish (*Carassius auratus*) at levels of 0, 20, 40, 60, 80, and 100% showed substitution level of 60% substitution provided optimal in growth, feed efficiency, and protease activity. However, increasing the proportion of black soldier fly larvae reduced efficiency across various parameters, suggesting the limits of high dosages on nutrient digestion and absorption, thereby reducing growth, as observed in catfish and rainbow trout (St-Hilaire et al., 2007; Anvo et al., 2017).

In contrast, the amylase and lipase activities increased with the increasing amounts of black soldier fly larvae replacement fish meal in diets that showed in the carp group fed diets that replaced fishmeal at 50 and 75% ($P<0.05$). The elevation of amylase activity is attributed to dietary adjustments in response to treatment, particularly the higher carbohydrate content from chitin in black soldier fly larvae, which exceeds that from fish meal. The black soldier fly larvae contain to 8.7 to 9.6% of chitin, regarded as an anti-nutritional factor in aquafeeds due to its indigestibility (Diener et al., 2009; Kroekel et al., 2012; Jayanegara et al., 2020; Shah & Hayat, 2024), whereas fishmeal contains less than 1% chitin, which is a very low level due to the absence of chitinous exoskeletons in fish (Pascon et al., 2025). It causes the increased release of digestive enzymes (amylase) for activity. The augmentation of lipase activity correlates with the elevated fat content in the proximate composition of the experimental food,

which increases as the replacement of black soldier fly larvae increases, leading to greater lipase release to facilitate fat digestion. In juvenile largemouth bass, a diet comprising up to 50% black soldier fly larvae elevated the levels of DHA, EPA, and C22:5n-3 in the liver, indicating that black soldier fly larvae could assist in the maintenance of these essential fatty acids (You et al., 2024). The fatty acid profile of black soldier fly larvae noticeably contrasts with that of fish oils containing long-chain EPA (20:5n-3) and DHA (22:6n-3). BSFL, however, has a substantial quantity of long-chain lipids, notably including lauric acid, as well as palmitic, myristic, oleic, and linoleic acids (Srisuksai et al., 2024; Dîrvari et al., 2025). Studying on goldfish (*Carassius auratus*) and eels (*Monopterus albus*) has demonstrated that an increase in dietary fat content elevates lipase enzyme activity (Hu et al., 2020; Kamalii et al., 2022). Moreover, Muslimin et al. (2023) reported elevated levels of amylase and lipase in cork fish (*Channa striata*) exposed to several proportions of black soldier fly larvae as treatments, specifically at 20, 50, 80, and 100%, which is likely attributable to this study.

The ingredients in food significantly impact on the colors of ornamental fish, particularly carotenoids. These compounds are mainly natural pigments that are abundant and enhance pigmentation, giving the fish different colors such as yellow, red, and orange, thereby influencing the fish's beautiful color (Saikia & Das, 2023; Rehman et al., 2023). The study found that the total carotenoid content of all treatment diets ranged from 0.39 to 1.41 $\mu\text{g/g}$. The color intensity of koi carp in this study exhibited no significant differences in L^* , a^* , and b^* values from the skin above the lateral line ($P>0.05$) among all groups. This result shows that the black soldier fly larvae level used as a replacement for fish meal in the diet is insufficient to influence the fish's coloration and aligns with the skin expression. According to Jintasataporn et al. (2005), the cultivation of fancy carp with diets containing different carotenoid levels, specifically 96.2 and 103.9 $\mu\text{g/g}$, resulted in an increased redness value on the fish skin compared to the control group ($P<0.05$). During the 15-day investigation, the a^* value exhibited an upward trend. In contrast, the b^* value decreased and stabilized by the end of the experiment as the fraction of black soldier fly larvae substitution in fishmeal increased ($P>0.05$). In contrast, The a^* value in rainbow trout fillet was negatively affected by the replacement of dietary black soldier fly larvae meal ($P>0.05$) in all groups (Renna et al., 2017). The incorporation of *Tenebrio molitor* larvae meal as a dietary substitute for fish meal in blackspot sea bream (*Pagellus bogaraceo*) resulted in a reduction of the a^* value in the dorsal skin region. It decreased both L^* and a^* values in the ventral skin area (Iaconisi et al., 2017). Khieokhajonkhet et al. (2002) investigated diets with varying concentrations of black soldier fly larvae meal in goldfish, specifically 0g kg^{-1} , 43g kg^{-1} , 84g kg^{-1} , 145g kg^{-1} and 210g kg^{-1} , as substitutes for fishmeal. The dietary inclusion of black soldier fly larvae meal resulted in a linear and/or quadratic reduction of a^* and b^* values in the head, as well as L^* , a^* , and b^* values in the abdomen, alongside a decrease in total carotenoid content in the fin, serum, and skin. The

results concerning color varied among different fish species when black soldier fly larvae were utilized as a substitute for fishmeal in aquatic animal feed. This study demonstrated that substituting fishmeal with black soldier fly larvae in carp feed did not influence the fish's coloration. Fish are unable to synthesize their own carotenoid pigments and must obtain them from their diet. Spirulina has been added into the experimental diet as an ingredient for ornamental fish to enhance color intensity through carotenoids. Numerous studies on ornamental fish have demonstrated its ability to increase pigmentation (Gouveia et al., 2003; Vernès et al., 2015). The water quality during the experiment fluctuated but remained within the acceptable range for fish culture (Svobodova et al., 1993). Therefore, the black soldier fly larvae could substitute for fish meal in the diet, and has no effect on the water quality of the cultural system.

Conclusion

This study demonstrates that black soldier fly larvae can partially replace fishmeal in koi carp feed. The extent of substitution is a pivotal factor affecting aquaculture. Integrating black soldier fly larvae into the diet at levels above 50% negatively impacts growth performance and feed efficiency, as evidenced by declines in final body weight, weight gain, and average daily growth rate, along with an increase in the feed conversion ratio. The findings indicate a notable decline in protease activity with increasing replacement levels, whereas amylase and lipase activities tend to rise as replacement rates increase. These modifications resulted from reduced protein and increased fat content in the diets that used black soldier fly larvae as a replacement for fishmeal in the trial. However, survival rates and skin pigmentation intensity were unaffected by the application of black soldier fly larvae, showing that it did not impact fish viability or aesthetic attractiveness. The research findings indicate that black soldier fly larvae can replace fish meal in koi carp diets at levels up to 25% without negatively impacting growth performance, digestive enzyme activity, or skin coloration, producing results similar to those of fish meal-based diets. This supports the use of black soldier fly larvae as a sustainable source of protein for koi carp.

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