



Using *Hermetia illucens* larvae to recycle two types of organic waste to produce a sustainable feed source for fish and evaluate its effect on some blood parameters

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Abstract

This study highlights the potential of using environmentally harmful organic waste to produce a sustainable, environmentally friendly feed source that can be produced locally and at a low cost, potentially reducing reliance on expensive traditional feed sources used in fish production. Therefore, this study aimed to evaluate the use of powder black soldier fly larvae (BSFLP) (*Hermetia illucens* L.) reared on two types of organic waste as substrates (kitchen waste and fish processing waste) in the diets of common carp and its effect on some blood parameters. A total of 75 fish were distributed into five experimental groups: the first group was given a control diet (without BSFLP), the second and third groups were given diets supplemented with BSFLP and were raised on kitchen waste at 8% and 16% of the feed components, respectively. The fourth and fifth groups were given diets supplemented with BSFLP and were raised on fish processing waste at 8% and 16% of the feed components, respectively. A significant increase ($P \leq 0.05$) in blood glucose levels was found in the groups in which the larvae were used compared to the control group. Total protein and albumin levels did not show any significant differences, while globulin levels were significantly superior in the third group. The results also showed a significant decrease in triglycerides in the second and third groups compared to the control group. There were no significant differences in cholesterol levels, ALT and AST enzymes, blood hemoglobin, packed cell volume, and Mean Corpuscular Hemoglobin Concentration across all experimental groups.

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Introduction

Many countries around the world are moving towards achieving sustainable development, preserving the environment, and making optimal use of their natural resources. On the other hand, the increasing global population and rising living standards have led to increased demand for animal protein sources (1,2). Fish are considered one of the main sources of high-quality animal protein (3,4). However, the aquaculture industry faces a major challenge

in providing the raw materials used in the manufacture of fish feed, as the cost of feeding represents about 60-70% of the total costs of fish production projects (5,6). To provide food sources and enhance food security in many countries of the world, especially developing countries, and to face future challenges associated with providing sufficient food for more than 9 billion people by 2050 (7). Researchers around the world have sought to find new and alternative feed sources that can be used in animal nutrition and reduce dependence on traditional sources, which can also be used in

human nutrition (8,9). One of the proposed alternatives is insects, which are considered a promising feed source (10) and are receiving increasing attention as an alternative food source (7). Insects are characterized by high productivity and a low carbon and water footprint. In addition to its rapid growth on a wide range of organic wastes (10). Black soldier fly larvae (BSFL) are an innovative model for these modern biological solutions, as they have demonstrated high efficiency in converting multiple types of organic waste (such as food scraps, agricultural waste, slaughterhouse waste, animal manure, and many other organic wastes) into biomass rich in important essential nutrients (11). BSFL is considered one of the most usable insect species as a non-traditional feed source that can be produced locally and is environmentally friendly (12). It is non-pathogenic, safe, and does not pose any health risk to humans or animals (13). Moreover, these larvae are very effective in converting low-value and environmentally harmful organic waste into highly nutritious and beneficial nutrients that can be used in animal feed (14). The amount of organic waste produced worldwide is estimated at more than 1.3 billion tons annually, the accumulation of this waste and its failure to dispose of it in a proper manner can cause environmental pollution and the spread of epidemics and diseases (15).

Black soldier fly larvae powder (BSFLP) contains high levels of protein and a good composition of important amino acids similar to that found in fish meal and even exceeding the essential amino acid composition found in soybean meal (16). (17) noted its high content of essential amino acids such as methionine, lysine, tryptophan, valine, histidine, and others. BSFL is also a rich source of important minerals such as phosphorus, calcium, sodium, potassium, magnesium, zinc, manganese, and others (18). It also contains many vitamins, most notably vitamins B, C, E, and D3 (19). The larvae are rich in fatty acids, particularly saturated fatty acids and medium-chain fatty acids, such as lauric acid C12:0, which is a good source of energy and can also act as an antibacterial (20). (21) also reported that BSFLP contain good levels of linoleic and linolenic acids, which are essential fatty acids important for many biological functions in the body. (22) confirmed the presence of chitin, which is an effective antioxidant and immune-enhancing substance and can be used as a feed additive in pig and poultry nutrition (23,24). In addition, larvae contain antimicrobial peptides (AMPs), known for their antibacterial, antiviral, and antifungal properties, making them one of the best natural alternatives to reduce the excessive use of antibiotics for animals (25,26). The chemical composition of larval powder and its nutritional content vary due to many factors, such as: the insect's age stage, the type of food it consumes and grows on, the nature of the available environmental factors, the method of killing and drying it, the duration and method of storage, and other factors (20).

As a result of all this information, this study was conducted to evaluate the use of BSFLP reared on two types of organic waste as substrates (kitchen waste and fish processing waste) in feeding common carp and its effect on some blood parameters.

Materials and methods

Ethical approve

All experimental procedures on fish were carried out in accordance with the guidelines issued by the Institutional Research Ethics Committee of the College of Veterinary Medicine, University of Mosul, Iraq: Ethical Approval No. (UM.VET.2024.138).

Rearing of larvae and production of BSFLP

The study was conducted in the Fish Laboratory of the Department of Animal Production, College of Agriculture and Forestry, University of Mosul, Iraq. First, the organic waste used to feed the larvae was prepared. Two types of waste were used:

- 1- Kitchen waste: This included all types of food scraps, such as bread, rice, cooked and spoiled vegetables, spoiled fruits, as well as spoiled and inedible foods, etc. This waste was obtained from a student cafeteria at the University of Mosul.
- 2- Fish processing waste: This includes the remains resulting from the slaughtering and preparation of fish meat, including all inedible parts such as fish viscera, skin, heads, etc. These were obtained from local fish markets.

After bringing the organic waste to the laboratory, each source was placed separately in a small plastic barrel (60 liters capacity), with approximately 5 kg of waste being placed in each barrel. Then, black soldier fly eggs were added, which were obtained from one of the centers specialized in breeding and producing black soldier fly in the Nineveh Agriculture Department, Iraq, at a rate of 10g of eggs in each barrel. Suitable environmental conditions were created for the egg hatching process, with a temperature of approximately $30\pm5^{\circ}\text{C}$ and a relative humidity of approximately $65\pm10\%$. After four days, the eggs began to hatch, and the hatched larvae began feeding directly on organic sources (11). Organic waste was added to the larvae daily as needed. This process continued regularly for seventeen days until the larvae reached the desired size. Afterwards, the larvae were harvested and separated from the waste using a special sieve. They were then placed in an oven to dry and remove excess moisture at 50°C for three days. After removing them from the oven, they were left to cool at room temperature, and then the dry larvae were turned into a fine powder using a grinding machine. Samples were then taken to the laboratory for chemical analysis of the BSFLP components, as shown in Table 1.

Study Design and Formulation of Experimental Diets

Seventy-five common carp (*Cyprinus carpio* L.) with an initial weight of 88.7 ± 0.4 g were distributed into five experimental groups, with three replicates for each group, and five fish in each replicate. The diet of all groups contained the same percentage of crude protein (27.3%) and metabolizable energy level (3000 kcal/kg feed), with differences in the inclusion levels of black soldier fly larvae powder among the groups. The five experimental diets were prepared individually as shown in Table (2). The feed components were ground with a grinding machine and BSFLP was added to the diets in the following proportions: Group One (G1): control diet without addition of BSFLP. Group Two (G2): Addition of BSFLP-K at 8% of the diet components. Group Three (G3): Addition of BSFLP-K at 16% of the diet components. Group Four (G4): Addition of BSFLP-F at 8% of the diet components. Group Five (G5): Addition of BSFLP-F at 16% of the diet components.

After the feed ingredients were thoroughly and evenly mixed, water was added until the desired moisture level was reached, and the mixture was placed in a 3 mm diameter pelletizer. After pelletizing, the pellets were air-dried with occasional stirring for 24 hours. They were then stored in plastic bags until ready to use.

Table 1: Chemical composition of BSFLP based on dry weight

Components	BSFLP-K%	BSFLP-F%
Protein	31.14	31.25
Fiber	10	10
Humidity	8.5	5.9
Carbohydrates	3.07	0.47
Ash	5.2	5.5
Fat	42.09	46.88

BSFLP-K: Black soldier fly larvae powder reared on kitchen waste. BSFLP-F: Black soldier fly larvae powder reared on fish processing waste.

Fish Processing and Management

The common carp was obtained from the Tajran Hatchery, which specializes in fry production and is located near the city of Erbil. Subsequently, the fish were transferred to the fish farming laboratory. All fish were thoroughly disinfected with a saline solution at a concentration of 3 g NaCl/L water (5). Fifteen glass aquaria (60 cm length $\times$ 40 cm width $\times$ 40 cm height) were used. The aquaria were then filled with dechlorinated water, prepared from a special tank within the laboratory to ensure water quality and stable physical and chemical properties. Each aquarium was equipped with an air pump to oxygenate the water, and the dissolved oxygen concentration was adjusted to maintain the ideal level of 1 ± 5 mg/L. A submersible heater was installed

in each aquarium to regulate the water temperature to $25 \pm 3^\circ\text{C}$. The pH was measured daily and ranged from 7.1 to 7.8, which is within the recommended limits (27). Approximately 25% of the aquarium water was replaced daily using the siphon method, and fresh water at the same temperature as the aquarium was added to maintain water quality and reduce waste accumulation. The fish underwent an initial 10 days acclimatization period before the start of the study to help them adjust to the new rearing conditions in the aquaria and to ensure their health and freedom from any diseases.

The daily feed quantity was calculated based on the average live weight of each fish, with the fish fed 3% of their weight. The feed quantity was re-estimated and adjusted every three weeks by weighing all the fish, extracting the average weight of each fish, and then calculating the required feed quantity. The feed was provided in two meals daily: the first in the morning at 8:00 a.m. and the second in the evening at 6:00 p.m. to ensure optimal feed intake and maximum utilization. The experiment lasted 12 weeks.

Table 2: Composition of the experimental diets used for feeding common carp

Feed material	G1	G2	G3	G4	G5
Maize	31.16	25	15	25.25	15
Soybean meal	48	41.15	33.7	41	33.5
BSFLP-K	0	8	16	0	0
BSFLP-F	0	0	0	8	16
Wheat bran	4	5.69	7.4	5.65	10.51
Fodder wheat	6.14	11.66	21.4	12	18.49
Protein center	5	5	5	5	5
Vegetable oil	4.2	2	0	1.6	0
salt	0.25	0.25	0.25	0.25	0.25
Pelt Stabilizer (Bentonite)	0.5	0.5	0.5	0.5	0.5
Calcium monophosphate	0.5	0.5	0.5	0.5	0.5
limestone	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100
Calculated Chemical Analysis					
C. Protein	27.3	27.3	27.3	27.3	27.3
M. E. (kcal / kg)	3000	3000	3000	3000	3000

BSFLP-K: Black soldier fly larvae powder reared on kitchen waste. BSFLP-F: Black soldier fly larvae powder reared on fish processing waste.

Sample Collection and Blood Parameters Measurement

At the end of the 12-week study, six fish from each group (two fish from each replicate) were randomly selected for blood sample collection. The fish were anesthetized by spinal cord injury. Blood samples were then drawn from the caudal vein behind the anal fin using 3-ml plastic syringes.

The blood samples were divided into two portions: The first portion of blood was placed in glass tubes containing an anticoagulant (heparin) for hematological analyses. The second portion was placed in anticoagulant-free plastic tubes to obtain serum. The tubes were kept tilted at room temperature for about three hours, after which they were placed in a centrifuge at 3000 rpm for 10 minutes, then the serum was drawn using a special pipette and placed in an Eppendorf tube, and stored at -10°C until further analysis.

Biochemical tests of blood serum were performed to determine the concentrations of glucose, total protein, albumin, triglycerides, cholesterol, and the enzymes ALT and AST using the Genotek Smart-150 biochemical analyzer (USA-made). Globulin was calculated by subtracting the albumin value from the total protein. Hematological tests were also conducted to measure hemoglobin (Hb) and packed cell volume (PCV) using a Horiba ABX Micros 60 analyzer (France-made). The mean corpuscular hemoglobin concentration (MCHC%) was also calculated using the following equation:

$$\text{MCHC\%} = (\text{Hemoglobin} / \text{PCV}) \times 100$$

Study Data Analysis

Data were analyzed using a completely randomized design (CRD) to measure the effect of experimental

treatments on the studied indicators. Statistical Analysis System SAS (28) was used to compare averages and check for significant differences between them.

Results

Glucose, Total Protein, Albumin, and Globulin

The results shown in Table 3 indicate a significant superiority ($P \leq 0.05$) in glucose levels for all groups that incorporated BSFLP into their diet compared to the control group. The results also showed that there were no significant differences in the levels of total protein and albumin among all groups. Regarding the level of globulin, the third group achieved a significant superiority compared to the control group, while the rest of the groups did not differ from the control group.

Triglycerides, Cholesterol, ALT, and AST Enzymes

The results listed in Table 4 show a significant decrease ($P \leq 0.05$) in blood triglyceride levels in the second and third groups compared to the control. Data analysis results indicated no significant differences in ALT, AST and cholesterol levels for all groups that were given BSFLP as part of their diet compared to the control group.

Table 3: The effect of incorporating BSFLP reared on two types of organic waste in common carp diets on blood serum glucose, total protein, albumin, and globulin levels (Mean $\pm$ S.E)

Groups		Glucose (mg/dL)	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
G1	Control	97.66 $\pm$ 0.50 ^c	5.34 $\pm$ 0.34	1.37 $\pm$ 0.04	3.97 $\pm$ 0.33 ^b
G2	BSFLP-K 8%	133.00 $\pm$ 2.31 ^{ab}	6.30 $\pm$ 0.24	1.17 $\pm$ 0.13	5.14 $\pm$ 0.17 ^{ab}
G3	BSFLP-K 16%	121.67 $\pm$ 7.43 ^{ab}	8.44 $\pm$ 1.24	1.10 $\pm$ 0.05	7.34 $\pm$ 1.24 ^a
G4	BSFLP-F 8%	138.00 $\pm$ 2.09 ^a	7.74 $\pm$ 1.61	1.70 $\pm$ 0.48	6.04 $\pm$ 1.13 ^{ab}
G5	BSFLP-F 16%	116.34 $\pm$ 10.18 ^b	6.40 $\pm$ 1.29	1.07 $\pm$ 0.04	5.34 $\pm$ 1.30 ^{ab}
Significant level		*	N.S.	N.S.	*

* Different letters within a column indicate significant differences between groups at ($P \leq 0.05$) level. SFLP-K: Black soldier fly larvae powder reared on kitchen waste. BSFLP-F: Black soldier fly larvae powder reared on fish processing waste.

Table 4: The effect of incorporating BSFLP reared on two types of organic waste in common carp diets on blood serum Triglycerides, Cholesterol, AST, and ALT (Mean $\pm$ S.E)

Groups		Triglycerides (mmol/L)	Cholesterol (mmol/L)	AST (IU/L)	ALT (IU/L)
G1	Control	2.06 $\pm$ 0.30 ^a	2.70 $\pm$ 0.21	88.50 $\pm$ 5.27	26.17 $\pm$ 3.09
G2	BSFLP-K 8%	1.24 $\pm$ 0.04 ^b	2.64 $\pm$ 0.34	77.34 $\pm$ 6.84	26.00 $\pm$ 4.05
G3	BSFLP-K 16%	1.07 $\pm$ 0.07 ^b	2.27 $\pm$ 0.66	93.67 $\pm$ 5.55	24.34 $\pm$ 1.46
G4	BSFLP-F 8%	2.10 $\pm$ 0.12 ^a	3.00 $\pm$ 0.31	91.00 $\pm$ 6.56	26.34 $\pm$ 0.89
G5	BSFLP-F 16%	1.57 $\pm$ 0.39 ^{ab}	2.64 $\pm$ 0.22	93.67 $\pm$ 5.85	27.00 $\pm$ 1.53
Significant level		*	N.S.	N.S.	N.S.

* Different letters within a column indicate significant differences between groups at ($P \leq 0.05$) level. BSFLP-K: Black soldier fly larvae powder reared on kitchen waste. BSFLP-F: Black soldier fly larvae powder reared on fish processing waste.

Packed Cell Volume, Hemoglobin, and Corpuscular Hemoglobin Concentration

Table 5 shows the effect of adding BSFLP to common carp feed on some blood parameters. The results showed no

significant differences in packed cell volume (PCV%), blood hemoglobin concentration (Hb) and mean cell hemoglobin concentration (MCHC%) among all groups.

Table 5: The effect of incorporating BSFLP reared on two types of organic waste in common carp diets on PCV %, Hb (gm/ 100ml), and MCHC % (Mean $\pm$ S.E)

Groups		PCV (%)	Hb (gm/ 100ml)	MCHC %
G1	Control	37.00 $\pm$ 1.16	12.34 $\pm$ 0.39	33.35 $\pm$ 0.01
G2	BSFLP-K 8%	35.67 $\pm$ 4.64	11.89 $\pm$ 1.55	33.33 $\pm$ 0.07
G3	BSFLP-K 16%	35.34 $\pm$ 2.67	11.78 $\pm$ 0.89	33.34 $\pm$ 0.04
G4	BSFLP-F 8%	38.67 $\pm$ 5.82	12.89 $\pm$ 1.94	33.33 $\pm$ 0.05
G5	BSFLP-F 16%	33.34 $\pm$ 2.41	11.12 $\pm$ 0.81	33.35 $\pm$ 0.04
Significant level		N.S.	N.S.	N.S.

*Different letters within a column indicate significant differences between groups at ($P \leq 0.05$) level. BSFLP-K: Black soldier fly larvae powder reared on kitchen waste. BSFLP-F: Black soldier fly larvae powder reared on fish processing waste.

Discussion

Glucose, total protein, albumin, and globulin

This increase in blood glucose concentration in the groups whose diet included BSFLP may be due to the presence of several carbohydrate-digesting enzymes in the composition of these larvae, especially amylase (29,30), which plays an important role in the process of starch digestion and the analysis of maltose into two glucose molecules, thus contributing to the availability of glucose in the blood. In addition, larvae are rich in lauric acid, a medium-chain fatty acid that is an effective source of energy in fish feed (31), which may contribute to raising blood glucose levels. As for the elevated globulin levels, this is likely due to the presence of antimicrobial peptides (AMPs) in BSFLP, most notably defensins and cecropins (25). These peptides constitute the first line of defense against microbes and also stimulate the immune system in fish (32). Since globulins are a component of immune proteins, the activation of the immune system consequently leads to an increase in their concentration in the blood (33). These results are consistent with the study by (34), who confirmed a significant increase in blood glucose levels when soybean meal was replaced with BSF larvae meal as a protein source in the diet of *Catla catla* fish at rates of 80 and 100%. They also observed a significant increase in glucose levels when soybean meal was replaced by 40% BSF larval meal in the diet of *Labeo rohita* fish. The results of this study differed from (35) when they indicated that there was a significant increase ($P \leq 0.05$) in the levels of total protein and albumin in the blood of Nile tilapia fish when replacing fish meal with BSF larvae powder at ratios of 33 and 100%. Their results also showed a significant decrease in the glucose level of the treatments compared to the control.

Triglycerides, cholesterol, ALT, and AST enzymes

The significant decrease in triglyceride levels in the blood of fish from the second and third groups may be due to the BSFLP containing high levels of medium-chain fatty acids, particularly lauric acid, which is used as an energy source instead of being stored in the body as fat (31). This rapid use leads to a reduction in the accumulation of triglycerides in body tissues. These results are consistent with what was reported by (36) who indicated that fish meal can be replaced by BSF larvae meal in Jian carp (*C. carpio* var. Jian) feeds by up to 100% without any significant difference in cholesterol concentration and ALT and AST enzyme levels. (37) also reported similar results when replacing soybean meal with defatted BSF larva meal in the diets of grass carp at levels of 0, 25, 50, 75, and 100%. They found no significant differences in ALT, AST or cholesterol among all treatments. (38) also noted no significant differences in glucose, ALT, and AST levels when BSF larval powder was partially or completely replaced by fish meal in the diets of juvenile mirror carp (*C. carpio* var. specularis). In contrast, the results of this study differed from those reported by (39) who observed no significant change in triglyceride levels, while there was a significant increase in cholesterol concentration when 75% of soybean meal was replaced with BSF larvae powder in common carp diets. (34) also indicated a significant increase in the level of triglycerides and AST enzyme when replacing soybean meal with BSF larvae powder in *Labeo rohita* fish feed at the ratios (80 and 100%), with a significant decrease in the level of ALT compared to the control.

Packed cell volume, hemoglobin, and corpuscular hemoglobin concentration

The use of BSFLP in fish feeding had no adverse effect on PCV%, Hb concentration and MCHC%. These results are

consistent with those reported by (40) when replacing fish meal with BSF larvae in Eurasian Perch Fish diets at ratios (0, 20, 40 and 60%), as the results showed no significant difference in Hb concentration and MCHC% for all treatments compared to the control. (41) also indicated that no differences in PCV% and Hb were recorded when using BSF larvae powder at a rate of up to 100% instead of protein concentrate in the diet of common carp. While the results of this study differed from those of (35) when replacing fish meal with defatted BSF larvae in Nile tilapia diets at ratios (0, 33 and 100%), they found an increase ($P \leq 0.05$) in Hb, PCV% and MCHC% for all treatments compared to the control. (34) also observed a significant decrease in Hb and MCHC% in blood when soybean meal was replaced with BSF larval meal as a protein source in the diet of *Catla catla* fish at ratios of 80 and 100%.

Conclusion

No harmful effects on the studied blood parameters were observed when BSFLP was used in the diet of common carp at levels up to 16% of the feed ingredients. This study may also contribute to raising awareness about the possibility of utilizing environmentally harmful organic waste and converting it into a high-nutritional-value feed through the rearing and production of black soldier fly larvae, thereby promoting the concept of a circular economy by recycling organic waste in a scientific and safe manner to produce a new feed source that can be produced locally and at low cost, reducing reliance on expensive conventional feed sources, which may lower fish production costs and increase profits. Accordingly, BSFLP represents a sustainable and environmentally friendly economic option.

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Conflict of interest

There is no conflict of interest.

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استخدام يرقات *Hermetia illucens* في إعادة تدوير نوعين من النفايات العضوية لإنتاج مصدر علفي مستدام للأسماك وتقييم أثره في بعض معايير الدم

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الخلاصة

هدفت هذه الدراسة الى تقييم استخدام مسحوق يرقات ذباب الجندي الاسود (*Hermetia illucens* L.) التي تم تربيتها على نوعين من المخلفات العضوية (مخلفات المطابخ ومخلفات جزر الأسماك) في علائق اسماك الكارب الشائع وأثر ذلك على بعض معايير الدم. إذ استخدم 75 سمكة وزعت على خمس مجاميع تجريبية: المجموعة الاولى أعطيت عليقة السيطرة (بدون اضافة BSFL)، والمجموعتين الثانية والثالثة أعطيت عليقتين مضاف اليهما BSFL تم تربيتها على مخلفات المطابخ بالنسبتين 8 % و 16% من مكونات العليقة على التوالي. أما المجموعتين الرابعة والخامسة أعطيت عليقة مضاف اليها BSFL تم تربيتها على مخلفات جزر الاسماك بالنسبتين 8 % و 16% من مكونات العليقة على التوالي. كشفت نتائج الدراسة عن وجود ارتفاع معنوي ($P \leq 0.05$) في مستوى كوكوز الدم للمجاميع التي استخدم فيها اليرقات مقارنة بمجموعة السيطرة. بينما لم تظهر مستويات البروتين الكلي والالبومين أي اختلاف كبير، اما بالنسبة للكلوبولين فقد اظهرت النتائج وجود تفوق معنوي لصالح المجموعة الثالثة. كما بينت النتائج وجود انخفاض واضح في الشحوم الثلاثية للمجموعتين الثانية والثالثة مقارنة بالسيطرة. ولم يكن هناك اختلافات مهمة في مستويات الكوليسترول وانزيمي ALT و AST و هيموغلوبين الدم وحجم خلايا الم المرصوفة ومعدل تركيز هيموغلوبين الخلية في جميع المجاميع التجريبية.