EFFECT OF REPLACING SOYBEAN MEAL WITH LARVAE MEAL OF BLACK SOLDIER FLY *HERMETIA ILLUCENS* IN GROWTH PERFORMANCE AND SOME BIOCHEMICAL BLOOD PARAMETERS OF COMMON CARP *CYPRINUS CARPIO* L.

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ABSTRACT

This study was conducted at the College of Agriculture/ Diyala University/ Department of Animal production to determine the effect of replacing soybean meal with black soldier fly *Hermetia illucens* larvae meal on the growth performance and some biochemical blood parameters of common carp Cyprinus carpio L. One hundred and five fish with an average initial weight of 27.46±0.14 g was used in this experiment. They were randomly distributed into five treatments (3 replicate/treatment, 7 fish/ replicate). The first treatments (T1 control group) was fed with standard diet without black soldier fly larvae meal (0% BSFLM), black soldier fly larvae meal were substituted for soybean meal in other experimental groups at 25%, 50%, 75% and 100% (T2, T3, T4 and T5), respectively. After 12 weeks of feeding, no significant differences (P>0.05) were found in feed conversion ratio (FCR) and protein efficiency ratio (PER) between control and experimental groups. However, T4 (75% BSFLM) showed significantly improved in mean of final body weight (FBW) and weight gain (WG) compared to the control and other experimental groups. The results of serum biochemical indices showed no statistically significant differences (P>0.05) in the levels of ALT (Alanine aminotransferase), Glucose, Triglycerides, LDL (low-density lipoprotein) and total protein (TP) between control and experimental groups. While AST (Aspartate aminotransferase) level of T4 and T5 was significantly higher (P≤0.05) than T3. As a result, the study suggests that black soldier fly larval meal can be a sustainable protein alternative to soybean meal at 75% without affecting growth performance or blood biochemical characteristics.

Key words: Alternative proteins, protein efficiency ratio, Specific growth rate, Relative growth rate.
*** Part of the Ph.D. dissertate of the 1st author.**

تأثير استبدال مسحوق كسبة فول الصويا بمسحوق يرقات ذبابة الجندي الأسود (*Hermetia illucens*) في أداء النمو وبعض معايير الدم الكيموحيوية في اسماك الكارب الشائع (*Cyprinus carpio* L.)

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المستخلص

أجريت هذه الدراسة في كلية الزراعة/ جامعة ديالى/ قسم الإنتاج الحيواني لمعرفة تأثير استبدال كسبة فول الصويا بمسحوق يرقات ذبابة الجندي الأسود *Hermetia illucens* في اداء النمو وبعض معايير الدم الكيموحيوية في اسماك الكارب الشائع *Cyprinus carpio* L.، تم استخدام 105 سمكة بمعدل وزن 27.46±0.14 غم، وزعت عشوائياً إلى خمس معاملات بواقع ثلاث مكررات لكل معاملة وسبعة اسماك لكل مكرر، غذيت المعاملة الأولى (معاملة السيطرة T1) بعليقة دون اضافة مسحوق يرقات ذبابة الجندي الأسود (0% BSFLM)، وتم استبدال كسبة فول الصويا في المعاملات التجريبية بمسحوق يرقات ذبابة الجندي الأسود بنسبة 25% و50% و75% و100% لكل من المعاملات (T2 وT3 وT4 وT5) على التوالي. وبعد 12 أسبوعاً من التغذية، لوحظ عدم وجود فروق معنوية (P>0.05) في نسبة التحويل الغذائي (FCR)، ونسبة كفاءة البروتين (PER) بين معاملة السيطرة وبقية المعاملات التجريبية. بينما أظهرت المعاملة T4 (75% BSFLM) تحسنًا ملحوظًا في معدل وزن الجسم النهائي (FBW) والزيادة الوزنية (WG) مقارنة بمعاملة السيطرة وبقية المعاملات الأخرى، وأظهرت المؤشرات الكيموحيوية في مصل الدم عدم وجود فروق ذات دلالة إحصائية (P>0.05) في مستويات أنزيم ناقلة أمين الأنين (ALT) والجلوكوز والدهون الثلاثية والبروتينات الدهنية منخفضة الكثافة (LDL) والبروتين الكلي (TP) بين معاملة السيطرة وبقية المعاملات التجريبية، بينما كانت مستويات إنزيم ناقلة أمين الاسبارتات (AST) في المعاملة T4 وT5 أعلى بكثير (P≤0.05) من المعاملة T3. خلُصت الدراسة إلى أن مسحوق يرقات ذبابة الجندي الأسود يمكن أن يكون بديلاً بروتينيًا مستدامًا لمسحوق كسبة فول الصويا بنسبة 75٪ دون التأثير على أداء النمو أو الخصائص الكيموحيوية للدم.

الكلمات المفتاحية: البروتينات البديلة، نسبة كفاءة البروتين، معدل النمو النوعي، معدل النمو النسبي.
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INTRODUCTION

Currently one of the important sources of animal protein consumed by humans is aquaculture, which in 2013 surpassed beef production (5, 22). Moreover, in 2018, human consumption of fish production exceeded 50% (13). Therefore, this development in aquaculture production in recent decades cannot be separated from fish nutrition (35). One of the major challenges in the future is to provide enough high-quality proteins to the rapidly growing aquaculture industry. One of the priciest feed ingredients that is included in the composition of fish diets is protein, as its levels range between 30-50% depending on the species and age stage of fish and for many years, fishmeal was the primary source of protein in fish feed, because it provides the majority of balanced amino acid requirements for fish nutrition, (2, 16, 48). In addition, the palatability and high digestibility of nutrients and lack of anti-nutritional factors make fishmeal an important source of protein in fish diets (15, 18). However, economic, environmental and social concerns have led to conducting many studies on the most sustainable alternative sources of protein compared to fishmeal (35). In recent years, terrestrial plants have gained great importance as alternative sources of protein in many studies, despite the fact that they negatively affect feed intake, digestion, absorption of nutrients, and fish health because they contain biologically active and anti-nutritional factors (15,18,16). The physical, chemical, and nutritional quality of aquafeed ingredients is also affected by the source of the raw materials used in formulating the diet, including production and harvesting costs, as well as the cost of preparing, storing, and transporting the final product, For example, if soybean meal is not properly heat treated during the manufacturing process, it will contain anti-nutritional factors such as trypsin inhibitors, and lectin is a carbohydrate-binding protein that makes up 10% of total soybean protein. On the other hand, overcooked soybean meal will lead to damage the amino acids especially lysine, which leads to decreased bioavailability, and thus a decrease in product quality as well (44). Soybeans are the main source of plant protein for livestock and fish feed at the global level and according to international reports, many countries in the world imported 164.32 million metric tons of soybeans for the period between 2022 and 2023. China was the largest importer among the countries, importing about 96 Million metric tons, while the total import of soybeans by other Asian countries reached 43 million metric tons, and the European Union imported about 14.4 Million metric tons of soybean meal (41). This heavy reliance on world soybean imports raises concerns about the potential vulnerability of its sustainable supply in the future, as well as ongoing concerns about the environmental repercussions associated with its production (40). On the other hand, soybeans has become less dependable as a sustainable source of protein due to competition between organisms and humans for sources of protein in food (39). Therefore, this necessitates investigating new protein sources that may be produced locally in a way that maintains a sustainable protein supply and reduces the costs and uncertainties associated with imports (16). Aquafeed production can take advantage of large quantities of organic products by converting them into biomass rich in proteins and lipids using living organisms (26, 38). Bioconversion through insects growing on organic by-products as a feed source may represent a valuable solution (6). It also represents a valid example of sustainable livestock production in terms of land use, water consumption, and CO_2 production due to the reduced energy requirements during rearing (43). The black soldier fly *Hermitia illucens* is a promising, harmless insect that contributes to solving part of the agriculture’s growing problems by being an alternative source of protein in animal feeds and eliminating organic waste and by-products (34). Tumpa et al. (46) showed that BSF larvae can convert low-quality organic waste into high-quality protein that can be used in fish feed production, allowing aquaculture production to remain economically and environmentally viable. Due to the lack of similar studies in Iraq, the black soldier fly may represent an opportunity in the economic insect industry that may contribute to reducing feeding costs in aquaculture, recycling organic waste, and providing a sustainable alternative to traditional protein sources. This study aims to investigate the possibility of replacing soybean meal with black soldier fly *Hermetia illucens* larvae meal on growth performance and some biochemical blood parameters of common carp *Cyprinus carpio* L.

MATERIALS AND METHODS

Experimental fish: The common carp *Cyprinus carpio* L. used in this study was purchased from a local fish farm (Baghdad/Mada'in - Iraq), and the fish were then subjected to a two-week acclimation period, fish were fed with control diet, to ensure optimal environmental quality. Water parameters were carefully monitored daily: dissolved oxygen (DO, mg/L), pH, temperature (°C) and total ammonia (mg/L), according to pre-established protocols.

Experimental design and diet formulation Experimental glass tanks with dimensions of 60 x 40 x 40 cm were prepared by washing and sterilizing them with coarse salt, and leaving them for 72 hours after washed, then filled with 72 liters of water and equipped with air pumps to ensure that the water were supplied with sufficient amounts of oxygen. The fish, with an average initial weight of 27.46±0.14 g, randomly divided into 15 experimental glass tanks in 5 treatments. Each treatment had three replicates (7 fish per tank). Five different diets formulated to replace soybean meal with black soldier fly larvae meal: T1 control group (0% BSFLM) diet without black soldier fly larvae meal inclusion, T2 (25% BSFLM) T3 (50% BSFLM), T4 (75% BSFLM), and T5 (100% BSFLM) In addition, the diets were balanced when replacing BSF larval meal (table 1). fish were fed for 90 days gradually, at 3% body weight at the beginning (first month), 4% at the end of the experiment. All five treatments of fish were fed diet three times daily, fish weight in each tank was determined every two weeks, and the amount of feed fed to the fish was adjusted accordingly**.**

Table 1. Ingredients used and proximate compositions measured in the five experimental diets.

Ingredients	T1 Control 0% (BSFLM)	T2 %25 (BSFLM)	T3 %50 (BSFLM)	T4 %75 (BSFLM)	T5 %100 (BSFLM)
Fish meal	10	10	10	10	10
Protein concentrate	20	20	20	20	20
Soybean meal (%46)	30	22.5	15	7.5	0
Wheat flour	10	10	10	10	10
Millet pearls	7	7	7	7	7
Yellow corn meal	11	11	11	11	11
Wheat bran	10	10	10	10	10
Plant oil	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5
Vitamins and minerals	1	1	1	1	1
*BSFLM	0	7.5	15	22.5	30
Total	100	100	100	100	100
Proximate chemical composition					
Crude protein	34.19	34.16	34.13	34.10	34.07
Crude fat	3.2	4.15	5.4	5.5	6.55
Fiber	7.4	6.6	6.9	7.3	7.8
Carbohydrate	42.12	41.08	40.28	39.61	38.87
Ash	8	7.5	7.5	7	7
moisture	5.09	6.51	5.79	6.49	5.71

*** BSFLM: Black Soldier Fly Larvae meal.**

Determination of growth performance

Weight gain (WG)

WG =W2– W1

W2= final weight

W1= initial weight

Relative growth rate (RGR) %

RGR = (final weight - initial weight) ÷ initial weight x 100

Specific growth rate (%/day) (SGR): It was calculated according to the equation mentioned by Mohammed and Al-Khshali (0033)

SGR %/day = {(Ln W2–Ln W1)/(T2–T1)}x 100

Ln W2 = the natural logarithm of the second weight at time T2

Ln W1 = natural logarithm of the first weight

Feed conversion ratio (FCR) it was calculated according to the equation mentioned by Al Khshali and Saleh (4)

FCR=amount of feed given (g)/weight gain (g).

Protein Intake (PI) (g):

PI (g) = Total feed consumed × Crude protein feed / 100

Protein efficiency ratio (PER)

PER = weight gain (g)/protein intake (g)

Blood serum parameters

At the end of the experiment, blood samples were taken from the caudal vein of three fish from each treatment and immediately centrifuged using the method described by Ma et al. (28). The serum was collected and stored at -40° C until further analysis; serums were prepared by centrifugation (3000 r/min, 4 °C) for 15 min. The AST (aspartate aminotransferase), ALT (alanine aminotransferase), Glu (glucose), Chol (total cholesterol), TG (triacylglycerol), HDL (high density lipoprotein), LDL (low density lipoprotein), TP (total protein), and ALB (albumin) were measured on a Semi-Auto Chemistry Analyzer, Mindray BA-88A, Reagents and test kits were purchased from Mindray.

Statistical analysis

After confirming the normality of the data with the Kolmogorov-Smirnov test, one-way variance analysis (ANOVA) and Duncan's multiple range test at a significant level of 0.05 were used for data analysis using SPSS Statistics software version 26.0. Values are represented as mean ± standard error.

RESULTS AND DISCUSSION

Growth performance: Results the effects of experimental diets on growth performance of common carp *Cyprinus carpio* L. presented in Table 2. showed no significant (P>0.05) effect in feed conversion ratio (FCR) and protein efficiency ratio (PER) among treatments after 90 days of feeding but, there are a significant differences (P≤0.05) in final body weight (FBW) and weight gain (WG) in T4 treatment (75%BSFLM) which was significantly (P≤0.05) higher than that of the control treatment (0% BSFLM), and other treatments Relative growth rate (RGR) and Specific growth rate (SGR), in the T4 (75%BSFLM) was significantly higher than T5 (100% BSFLM) and T3 (50% BSFLM). This study showed that the growth performance of common carp fed diets with BSFLM replacing 75% of soymeal were significantly different compared to the control and other treatments. The improvement in growth performance in T4 may be attributed to the fact that the diet in T4 was balanced in terms of composition and content of essential amino acids and fatty acids, and that this combination increased the palatability and acceptance of this diet by fish, which was reflected positively in growth characteristics in final body weight (FBW) and weight gain (WG). Previous nutritional tests have shown that BSFL meal is high in protein, necessary amino acids, and healthy fatty acids (**Error! Reference source not found.**5) Similar results have been found in grass carp *Ctenopharyngodon idellus* (27, **Error! Reference source not found.Error! Reference source not found.**0019), and Nile tilapia *Oreochomis niloticus* (0034). Linh et al. (25) have reported that black soldier fly larval meal can be effectively used as a protein substitute in koi carp *Cyprinus carpio* var. *koi* diets and aquafeed formulations, which has a positive effect on growth indicators and immune-related gene expression. In this study, the growth performance of common carp was significantly decreased when the BSFLM substitute for soymeal reached above 75% level. As a result, increasing or decreasing the amount of soybeans replaced with BSFLM above 75% may be detrimental to the growth of common carp fish, as the lower percentage may not meet the fish's basic nutritional requirements for protein, fat, carbohydrate, and ash, while high replacement rate of BSFLM above 75% in fish diet may cause physiological stress due to increased energy expenditure to eliminate excess nutrients or possibly due to high chitin content. A similar result founded in Atlantic salmon *Salmo salar* when the BSFLM inclusion levels in the diet exceeded 33% (14). On the other hand, BSFL meal replacement significantly reduced growth performance in juvenile Siberian sturgeon *Acipenser baerii* (**Error! Reference source not found.**0008).

Table 2. Effect of BSFLM on growth performance indices of the experimental fish

index	T1 Control 0% (BSFLM)	T2 25% (BSFLM)	T3 50% (BSFLM)	T4 75% (BSFLM)	T5 100% (BSFLM)
	Experimental groups				
IBW (g)	27.38±0.13	27.38±0.09	27.48±0.09	27.57±0.01	27.48±0.09
FBW (g)	76.52±1.59 b	76.62±0.55 b	75.62±2.03 b	81.43±1.15 a	75.24±1.16 b
WG (g)	49.14±1.67 b	49.24±0.53 b	48.14±1.95 b	53.86±1.15 a	47.76±1.19 b
RGR %	179.47±6.62 ab	179.84±2.04 ab	175.18±6.62 b	195.36±4.19 a	173.80±4.54 b
SGR (% day^{-1})	1.14±0.03 ab	1.14±0.01 ab	1.12±0.03 b	1.20±0.02 a	1.12±0.02 b
FCR	2.65±0.12	2.54±0.09	2.63±0.10	2.55±0.01	2.74±0.07
FI (g/fish)	130.06±1.87 ab	125.07±4.40 b	126.49±0.58 b	137.23±2.50 a	130.75±0.78 ab
PI	44.47±0.64 ab	42.72±1.50 b	43.17±0.20 b	46.80±0.85 a	44.55±0.27 ab
PER	1.11±0.05	1.15±0.04	1.12±0.04	1.15±0.01	1.07±0.03

*** Values are means and standard error. Values with different letter in the same row are significantly different (P ≤ 0.05) from each other.**

* BSFLM= Black Soldier Fly Larvae meal, IBW=Initial Body Weight, FBW=Final Body Weight, WG=Weight Gain, RGR %= Relative growth rate, SGR=Specific Growth Rate, FCR=Feed Conversion ratio, FI= feed intake, PI= Protein Intake, PER= Protein efficiency ratio. The differences in growth performance reported by different studies may be due to different nutrient compositions in BSFL meal, different tolerance of BSFL meal components such as chitin between fish species, different life stages of fish used in the experiments and different BSFL meal protein processing methods (00019). According to Karlsen et al. (0020) there was a negative correlation found between the digestibility coefficients of nutrients such as protein, fat and chitin concentration, which could explain the detrimental impacts on growth performance associated with increasing inclusion of BSFLM. However, Nguyen et al.**0** (0036) reported the additions of plant protein in high level to fish diets often lead to reduced growth due to high crude fiber content, poor palatability, decreased fat digestibility, presence of anti-nutrients factors and the imbalance of essential amino acid all that decreased daily weight gain. The growth-inhibitory effect of soybean meal has been associated with anti-nutritional factors, which reduce nutrient bioavailability, palatability, and digestibility due to excessive levels of non-soluble fiber and starch (10). BSF larval meal is commonly used in aquaculture due to its high nutritional value, which is rich in protein and fat, and the content of essential amino acids in BSFL meal is similar to that found in fishmeal meal rather than in soybean meal, which is a commonly used vegetable protein source (012). Therefore, Protein and amino acid profile of BSF larval meal may be more suited for fish (0000030, 00450).

Serum biochemical indices

Blood biochemical indicators are widely used for determining the nutritional status, health status, and adaptability of fish to the external environment (070). In addition, these indicators are essential in aquaculture nutrition, evaluating the effectiveness of compound feeds, physiological stress, health performance, and fish growth (029,0 11). The serum biochemical index results in Table 3 are shown that the dietary treatments did not differ significantly (P>0.05) in the levels of ALT, Glucose, Triglycerides, LDL (low-density lipoprotein) and total protein (TB) between control and experimental treatments. However, AST (Aspartate aminotransferase) level of the treated groups T4 (75%BSFLM) and T5 (100%BSFLM) was significantly higher (P≤0.05) than T3 (50%BSFLM) group but did not differ significantly with T1 control treatment (0%BSFLM) and T2 (25%BSFLM). higher levels of AST or ALT in the blood suggest damage to the liver cells as a mark of liver necrosis when they increase (47, 00006).

The secretion of liver enzymes and the destruction of body cells increases when the level of free radicals is greater than that of antioxidants (**Error! Reference source not found.**32). In the present study, common carp showed elevated blood AST activity when the replacement level of BSFL meal exceeds 75% in the experiment diet. However, the changes in AST values were not in a way that indicates negative effects on liver and pancreas health. Li et al. (023) found histopathological changes and mild hepatic necrosis in the liver of Jian carp fed a defatted BSF larvae meal at a replacement level exceeding 75%. Moreover, these effects was mild and did not lead to significant differences in liver enzyme indicators. Cholesterol level of T4 (75%BSFLM) was significantly higher (P≤0.05) than T1 control treatment (0%BSFLM), T2 (25%BSFLM), and T5 (100%BSFLM). The increase in this parameter seen in our study could be attributed to the fat content of larvae meal over soybean meal (021).

Table 3. Effect of BSFLM on blood serum indices of the experimental fish

index	Experimental groups T1 Control 0% (BSFLM)	T2 25% (BSFLM)	T3 50% (BSFLM)	T4 75% (BSFLM)	T5 100% (BSFLM)
ALT (IU/ml)	76.67±2.60	80.67±0.88	86.00±1.15	77.00±4.62	79.00±10.39
AST (IU/ml)	65.67±0.88 ab	62.00±2.31 ab	61.00±1.15 b	67.00±1.15 a	67.00±1.73 a
Glu (mg/dL)	91.67±2.03	88.67±0.88	88.00±5.77	84.00±8.08	86.00±2.31
Chol (mg/dL)	114.00±1.73 b	136.67±7.80 b	161.00±24.25 ab	189.67±6.06 a	135.67±17.61 b
TG (mg/dL)	165.67±4.33	208.67±17.61	217.00±32.91	164.67±4.91	222.67±12.99
HDL (mg/dL)	42.67±1.45 b	44.67±2.03 ab	45.67±1.45 ab	48.00±0.58 a	41.67±0.33 b
LDL (mg/dL)	83.67±2.60	85.67±2.03	81.67±0.33	83.00±2.89	84.00±0.58
TP (g/dL)	3.43±0.26	2.70±0.30	2.60±0.12	2.70±0.35	3.50±0.40
ALB (g/dL)	1.20±0.06 a	0.80±0.06 ab	1.35±0.14 a	0.97±0.43 ab	0.40±0.06 b

*** Values are means and standard error. Values with different letter in the same row are significantly different (P < 0.05) from each other.**

*** BSFL= Black Soldier Fly Larvae, AST= aspartate aminotransferase, ALT= alanine aminotransferase, Glu= glucose, Chol= total cholesterol, TG = triacylglycerol, HDL= high-density lipoprotein, LDL= low-density lipoprotein, TP= total protein, and ALB= albumin**

In this study, the cholesterol-lowering effect was not observed, which is attributed to the cholesterol-lowering properties of chitosan derived from chitin, which occurs at high levels of BSFLM (0042). This may be due to lower chitinase activity. As a result, the fish are unable to digest and absorb enough chitin or functional derivatives to induce a hypocholesterolemic effect as expected 0029). Furthermore, cholesterol, triacylglycerol, HDL (high-density lipoprotein), LDL (low-density lipoprotein), are important biochemical indicators of lipid metabolism status (9). On the other hand, some studies found that utilizing defatted BSFL meal to replace up to 75% of fishmeal in the diet had no negative influence on health-related serum biochemical indicators in Atlantic salmon *Salmo salar*, African catfish *Clarias gariepinus* and European sea bass *Dicentrarchus labrax* diets (6,00003,01). As for total protein levels in blood serum, there were no significant differences (P>0.05) between the experimental groups and there was no specific trend for the fish's ALB levels. However, T3 group (50% BSFL) recorded highest ALB level, Total serum proteins serve a variety of essential activities in fish (**Error! Reference source not found.Error! Reference source not found.**31). Albumin helps to maintain osmotic balance, transfer chemicals, and scavenge free radicals (00017). In addition, ALB and GLOB help to maintain the healthy system and function of plasma carriers (037), and ALB is an indication of hepatocyte damage and liver necrosis (00024).

CONCLUSIONS

In this study, the best results in growth performance of common carp showed when replacing 75% of soybean meal with black soldier fly larvae meal, which may be attributed to the good profile of amino acids in the larvae, high palatability, and good content of protein and fat. In addition, insects represent a natural food for many fish. On the other hand, the study found no significant changes in biochemical blood indicators that suggest a disease or immune disorder. In addition, study showed the possibility of using dried black soldier fly larvae as an alternative source of protein in the diet of common carp fish at rates not exceeding 75% without negative.

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