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Optimizing Broiler Performance: Evaluating Hemoglobin, Blood Chemistry, and Immune Response with Graded Levels of Dietary Black Soldier Fly Larvae

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Abstract:

Edible insects, a diverse class of arthropods, provide nutritional sustenance as part of traditional diets worldwide. Their consumption offers a substantial source of protein, vitamins, and amino acids. This experiment aimed to explore the optimal dietary inclusion level of black soldier fly larvae (BSFL) in broiler diets while evaluating their potential impact on animal health and immune response. The study focused on assessing various blood parameters, including Hemoglobin, RBC, WBC, PCV as well as liver and renal function markers (AST and ALT enzymes) alongside antibodies against Newcastle disease virus (NDV) and Kumboro disease. A completely randomized design was employed in a 35-day trial conducted within the university's poultry facility. Two hundred and twenty-five unsexed, day-old broiler chicks (Ross 308) were randomly distributed across five dietary treatments (n=3 replicates/treatment, n=15 birds/replicate). The treatments included: T1: Control - Commercial broiler starter diet (no additions) T2: 25% replacement of imported protein concentrate with BSFL larvae T3: 50% replacement of imported protein concentrate with BSFL larvae T4: 75% replacement of imported protein concentrate with BSFL larvae T5: 100% replacement of imported protein concentrate with BSFL larvae. The results revealed a significant increase in blood hemoglobin levels within treatments T3 (11.733), T4 (11.4433), and T5 (11.9300) compared to the control (T1) and T2 groups. Notably, other measured blood parameters (RBC, WBC, PCV, Heterophils, and H/L ratio) were not significantly affected by the dietary treatments, with all observed values remaining within the established physiological range. Additionally, the study found no significant differences in the concentrations of AST and ALT enzymes, uric acid, and creatinine across the various treatments with BSFL inclusion compared to the control group. Interestingly, a significant increase in Kumboro disease antibody levels was observed in comparison to the control (T1) and the 25% BSFL inclusion (T2) groups.

Keywords: Broiler, Insects, Black soldier fly larvae, enzymes, Humoral immune response, kumboro.

تحسين أداء فروج اللحم: تقييم الهيموغلوبين، صفات الدم الكيميائية والاستجابة المناعية باستخدام مستويات متدرجة من مسحوق يرقات ذبابة الجندي الأسود في العليقة.

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الخلاصة

توفر الحشرات الصالحة للأكل، وهي فئة متنوعة من المفصليات، غذاءً مغذيًا كجزء من الأنظمة الغذائية التقليدية حول العالم. ويوفر استهلاكها مصدرًا كبيرًا للبروتين والفيتامينات والأحماض الأمينية. هدفت هذه التجربة إلى استكشاف المستوى الأمثل لإدراج مسحوق يرقات **ذبابة الجندي الأسود (BSFL)** في علائق فروج اللحم، مع تقييم تأثيرها المحتمل على صحة الطيور والاستجابة المناعية. وركزت الدراسة على تقييم العديد من **معايير الدم**، بما في ذلك الهيموغلوبين، خلايا الدم الحمراء(RBC) ، خلايا الدم البيضاء (WBC) ، حجم الخلايا المكدسة (PCV)، بالإضافة إلى مؤشرات وظائف الكبد والكلى إنزيمات AST وALT، إلى جانب الأجسام المضادة لفيروس مرض نيوكاسل (NDV)ومرض الجامبورو.

تم استخدام التصميم العشوائي الكامل في تجربة استمرت 35 يومًا، وقد أجريت في قاعة الدواجن في جامعة البصرة كلية الزراعة. وتم توزيع مائتين وخمسة وعشرين من افراخ فروج اللحم (روز 308) غير محددة الجنس بعمر يوم واحد وبشكل عشوائي على خمس معاملات غذائية وبواقع (3 مكررات/معاملة، 15 طائر/مكرر). شملت المعاملات ما يلي:

- **T1**مجموعة السيطرة - علف بادئ تجاري لفروج اللحم (بدون إضافات).
- **T2**استبدال 25% من المركز البروتيني المستورد بمسحوق يرقات ذبابة الجندي الأسود.
- **T3**استبدال 50% من المركز البروتيني المستورد بمسحوق يرقات ذبابة الجندي الأسود.
- **T4**استبدال 75% من المركز البروتيني المستورد بمسحوق يرقات ذبابة الجندي الأسود.
- **T5**استبدال 100% من المركز البروتيني المستورد بمسحوق يرقات ذبابة الجندي الأسود

وكشفت النتائج عن زيادة معنوية في مستويات الهيموغلوبين في الدم ضمن المعاملات (11.733) T3 ، (11.4433) T4، و T5 (11.9300)مقارنة بمجموعتي السيطرة (T1) و T2 لوحظ أن معايير الدم الأخرى التي تم قياسها RBC ، WBC، PCV، الخلايا العدلة Heterophils ونسبة H/L لم تتأثر بشكل معنوي بالمعاملات الغذائية، حيث بقيت جميع القيم المرصودة ضمن النطاق الفسيولوجي الطبيعي.

بالإضافة إلى ذلك، لم تجد الدراسة فروقًا معنوية في تركيزات إنزيمات AST وALT، وحمض اليوريك، والكرياتينين عبر المعاملات المختلفة التي تحتوي على يرقات الذبابة الجندية السوداء مقارنة بمجموعة التحكم. ومن المثير للاهتمام، لوحظت زيادة معنوية في مستويات الأجسام المضادة لمرض الجامبورو مقارنة بمجموعة التحكم (T1) ومجموعة الإدراج 25% من يرقات الذبابة الجندية السوداء(T2)

الكلمات المفتاحية: دجاج التسمين، الحشرات، يرقات ذبابة الجندي الأسود، الإنزيمات، الاستجابة المناعية

Introduction

In the burgeoning tapestry of sustainability, we are confronted with an urgent quest: to mitigate the ecological footprint of animal protein production. As we navigate this challenge, the lexicon of alternative protein sources is expanding, beckoning us to explore the untapped potential of protein-rich insects. By embracing this novel paradigm, we can simultaneously alleviate the strain on conventional protein sources like soybean meal, fostering a healthier planet and a more cost-conscious approach to nourishing our populace (Smetana *et al.*, 2023). Poultry is no exception to this rule, as chickens are known to forage for worms and larvae in the topsoil and litter as they roam. These tiny morsels hold a treasure trove of nutritional wealth within their unassuming forms. On a dry matter basis, insects boast an impressive 30% to 70% protein content (Churchward-Venne *et al.*, 2017), a testament to their bountiful amino acid reserves. Beyond their protein prowess, insects also harbor a wealth of fats, minerals, and vitamins (Aiking and de Boer, 2019), making them a nutritional powerhouse that can't be ignored. Among the different insect species, the black soldier fly (BSF) has emerged as a revolutionary insect with immense potential to reshape the

poultry industry and promote sustainable practices. Beyond their resemblance to the common housefly, BSF larvae boast an impressive nutritional profile, containing a remarkable 40-60% crude protein content on a dry matter basis (Purkayastha and Sarkar, 2021). This protein is rich in essential amino acids, mirroring the ideal profile required by poultry for optimal growth and development (Lu *et al.,* 2022). Additionally, BSF larvae are a treasure trove of essential minerals, boasting high levels of calcium, phosphorus, and magnesium, further augmenting their nutritional value for poultry diets (Tomberlin and Van Huis, 2020).

The true brilliance of the BSF lies in its remarkable ability to upcycle waste. These industrious insects act as bioconversion machines, transforming organic waste streams, including food scraps, manure, and even biosolids, into a protein- and nutrient-rich biomass (Kumar *et al.,* 2021). This not only diverts organic waste from landfills, reducing greenhouse gas emissions and environmental pollution, but also creates a valuable and sustainable feed source for poultry (Veldkamp *et al.*, 2021). The economic impact of BSF larvae in poultry production is equally promising. Studies suggest that incorporating BSF larvae meal into poultry diets can significantly reduce feed costs while maintaining, or even enhancing, broiler performance (Cullere *et al.*, 2016). This translates to increased profitability for poultry farmers and fosters a more circular and resource-efficient food system. In conclusion, the black soldier fly offers a compelling solution for the poultry industry. Its exceptional protein content, rich amino acid profile, and ability to upcycle waste position BSF larvae as a sustainable and cost-effective feed ingredient. As research continues to explore the full potential of BSF, this innovative approach holds immense promise for a more sustainable and secure future for poultry production. The study engaged in a systematic investigation involving two hundred twenty-five broiler, categorized into quintets, each subjected to a gradational inclusion of black soldier fly larvae (BSFL) in their dietary regime—ranging from nil to complete substitution of traditional protein concentrates. Hematological indices, encompassing hemoglobin, packed cell volume (PCV), cholesterol, glucose levels, as well as hepatic and renal function markers, were meticulously monitored. Concurrently, serological screening for antibodies against specific avian pathogens was conducted. The objective was to ascertain the optimal incorporation rate of BSFL that would concomitantly bolster health and immunological robustness in the subjects.

Materials and methods

Experimental design: This experiment was undertaken within the poultry unit belonging to the Department of Animal Production, Faculty of Agriculture, Basra University. The timeframe spanned from March 21st, 2023, to April 24th, 2023. A total of 225 unsexed, day-old chicks (Ross 308 breed) with an average weight of 40 grams each were utilized.

A completely randomized design (CRD) was employed for the experiment. The chicks were randomly allocated into five treatment groups, with three replicates per treatment and 15 birds housed in each replicate. A cage system (three tiers) was used for housing, with each cage accommodating 15 chicks. Throughout the 35-day duration, from the first day until slaughter, the chicks were fed the designated experimental diets. The chicks were fed a starter diet containing 23.13% crude protein and 2918 kcal/kg metabolizable energy for the first three weeks. A grower diet containing 19.23% crude protein and 3189 kcal/kg metabolizable energy was used from 4 to 5 weeks of age. The health and preventive program for chickens was implemented to ensure the health of the flock, as shown in Table 1 below.

Table (1) Feedstuff and chemical composition of the experimental diets.

Feedstuff composition (%)	Treatment				
	T1	T2	T3	T4	T5
Maize	43	43	43.1	43	43.1
Wheat	16	16	16	16	16
Soybean meal	33	33	33	33	33
Concentrate protein	4	3	2	1	0
BSF larvae meal	0	1	2	3	4
Vegetable oil	0.5	0.5	0.4	0.5	0.4
Premix (Vitamin-mineral mixture)	1	1	1	1	1
CaCO3	2	2	2	2	2
Salt	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100
Metabolizable energy (kcal)	3.63.15	3090.54	3117.91	3145.30	3172.68
(%) Crude protein	19.46	19.44	19.42	19.39	19.37
Crude fiber (%)	7.11	7.43	7.76	8.09	8.42
Fat (%)	3.31	3.79	4.27	4.75	5.24
Ash (%)	8.48	7.96	7.44	6.92	6.4

Blood parameters evaluation: Upon completion of the experiment, six birds per group were euthanized (two birds per replicate). Blood samples were centrifuged at 3000 rpm for 15 minutes to obtain serum. The serum was then utilized to measure the concentrations of total protein, albumin, triglycerides, uric acid, ALT, and AST through spectrophotometry using diagnostic standard kits provided by Biolab technical.

Measurement of the quantitative standard of antibodies against Kumboro disease using the ELISA test: Blood samples were collected from the femoral vein of 35-day-old birds, with 3 birds from each treatment group. Blood was drawn into 10 ml EDTA-coated vacutainer tubes (3 tubes per treatment). The tubes were refrigerated at 5°C overnight, and then serum was separated by centrifugation at 3000 rpm for 15 minutes. Serum samples were stored frozen at -20°C until further analysis. An ELISA (Enzyme-Linked Immunosorbent Assay) was used to quantify the antibody titer against Kumboro disease. ELISA is a highly sensitive technique that can detect and quantify antibodies in a sample. The sensitivity of the ELISA test is 0.0005 micrograms/ml, which is much higher than other serological tests such as HI (Hemagglutination Inhibition) with a sensitivity of 0.1, HA (Hemagglutination test) with a sensitivity of 0.01, and AID (Agar Immune Diffusion) with a sensitivity of 30 mg/ml.

Results and Discussion

Blood parameters: Table (2) shows the effect of BSF larvae powder substitution of protein concentrate in broiler in some parameters at the age of 35 days. It is noted from the results of the statistical analysis that there are no significant differences between the experimental parameters of

both red and white blood cells, pcv, Heterophilic cells, lymphocytes and the ratio of heterophilic cells to lymphocytes. While a significant increase ($p \leq 0.05$) in the hemoglobin HB concentration was observed in treatment T3, T4 and T5, which recorded the highest values sequentially, reaching 11.7333, 11.4433 and 11.9300 compared to control T1 and T2, which recorded 10.2067 and 10.3633 respectively.

A preliminary reading of the results of the cellular blood parameters suggests that the birds did not suffer from any stress or disease. Cellular blood characteristics are valuable indicators of the general health and physiological status of animals, including broilers. Among these characteristics, hemoglobin (HB) is of particular importance because it directly reflects the blood's ability to carry oxygen (Kim *et al.*, 2022). The improvement in HB values may be attributed to the improved metabolism resulting from the addition of BSFLM.

The results of the current study are consistent with those of Ayuningtyas *et al.* (2023) who replaced meat with BSF larvae meal at levels of 0, 5, and 7.5%. They observed that broilers fed the 7.5% treatment had the highest hemoglobin level compared to the other treatments. The results of this study also agree with Ayuningtyas regarding the concentrations of red blood cells, white blood cells, heterophils, and lymphocytes. Ayuningtyas found that these values were within the normal physiological ranges, indicating that the addition of BSFLM did not have a negative effect on the health and physiological status of the birds.

Outcomes of the present research are also in line with those of Mat *et al.* (2022). They observed significant differences in favor of the treatment with the highest BSFL content (12%) compared to the control treatment (0%) and the other treatments (4, 6, and 8%). The results of this experiment are also consistent with those of Kinasih *et al.* (2018), who observed the highest HB value in the group fed 20% BSFL and 3% propolis. They attributed this to the improvement in iron utilization. Data obtained in this study are consistent with those of Mohammed *et al.* (2017) , who replaced fishmeal with BSFLM at levels of 0 and 33% in broiler diets. They reported that the 33% BSFLM treatment had significantly higher HB values than the control treatment. The researchers attributed this superiority to the positive effect of the feed ingredients on the health status of the birds. However, the results of this study did not agree with those of Mohammed et al. (2017) regarding the concentrations of PCV and RBC. Mohammed et al. (2017) found that these values were significantly higher in the 33% treatment compared to the control treatment.

Biochemical blood measurements

Table 3 shows the effect of adding BSF larvae meal as a protein concentrate in broiler diets on the concentration of some biochemical parameters (total protein, albumin, globulin, glucose, and cholesterol). The results of the statistical analysis showed no significant differences in the concentrations of total protein, globulin, cholesterol, and glucose. These results are consistent with those of Raju *et al.* (2023), who found no significant differences in the concentrations of total protein and cholesterol when BSFLM were included at levels of 0, 2.5, and 5% in broiler diets.

The results of this study are also in agreement with those of Attivi *et al.* (2020), who found no significant differences in the concentration of total protein between the control treatment and all experimental treatments when maggot meal was replaced with fishmeal at levels of 0, 25, 50, 75, and 100% in broiler diets. Similarly, Schiavone *et al.* (2017) found no significant differences between treatments in the concentrations of total protein and cholesterol when soybean oil was replaced with BSLF larvae fat at levels of 50 and 100% in broiler diets.

Table (2). the effect of BSF larvae powder substitution of the protein concentrate in the broiler feed in some broiler hematological parameters mean ± SD.

Treatments	RBC ($ml/10^6$)	WBC ($ml^3/10^3$)	HB (g/dl)	PCV (%)	Heterophil (%)	Lymph (%)	H/L
T1	2.4433 ± 0.07219	21.1100 ± .200330	10.2067^{b} ± .323800	30.7333 ± .753580	26.9333 ± 1.67564	60.1867 ± 1.21867	.4490 ± 0 .036950
T2	2.6467 ± .028480	21.6033 ± .095280	10.3633^{b} ± .539670	31.5367 ± 1.05493	26.4833 ± 1.81437	60.2567 ± .965420	.4406± 0 .037010
T3	2.8233 ± 0.19462	21.5800 ± .465440	11.7333^{a} ± 0.22696	31.5900 ± 1.44431	30.8800 ± 1.64265	57.7400 ± 1.44922	.5361 ± 0 .037240
T4	3.0233 ± .222590	22.0067 ± .354320	11.4433^{ab} ± .527010	32.4100 ± 1.63808	30.4133 ± 1.87644	61.2200 ± 2.62718	.5013± 0 .052730
T5	2.9833 ± .363380	21.9400 ± .508360	11.9300^{a} ± .195020	31.9433 ± 1.08555	31.2833 ± 1.58491	58.4800 ± 1.52588	.5370 ± 0 .040680
significant	N. S	N. S	*	N. S	N. S	N. S	N. S

N.S None Significant: * Significant (vertically different letters represent significant differences at the level of $0.05 \geq P$) T1= control treatment without additives, T2= 25% replacement of (BSF-PC) imported protein concentrate T3, = 50% replacement of (BSF-PC) replacing the imported protein concentrate, T4= 75% replacement of (BSF-PC) replacing the imported protein concentrate, T5= 100% replacement of (BSF-PC) replacing the imported protein concentrate.

The results of this study are also consistent with those of Marono *et al.* (2017) , who found that replacing BSFLM larvae with soybean meal in laying hen diets from 24 to 45 weeks of age had no effect on the concentration of total protein and glucose. However, (Marono et al., (2017) found significant differences in the concentration of globulin and cholesterol, with the BSFLM treatment having a higher concentration of globulin than the soybean meal treatment, and the soybean meal treatment having a higher concentration of cholesterol. The results of this study differ from those of Mat *et al.* (2022) with respect to the concentration of total protein. Their results showed a significant increase in the treatment with 12% BSFLM inclusion compared to 0, 4, 6, and 8% in broiler diets.

Treatment T4 had a significantly higher ($P < 0.05$) albumin concentration of 2.7033 compared to the other treatments, which had albumin concentrations of 2.2800, 2.3967, 2.3600, and 2.1867 for

the first, second, third, and fifth treatments, respectively. Albumin is undoubtedly an important indicator of poultry health. Serum albumin is of paramount importance in maintaining broiler health and can be said to be a vital carrier of a variety of substances, including drugs and nutrients. It also helps maintain colloid osmotic pressure in the bloodstream and is actively involved in the transport of ions and charged molecules. In addition, albumin has the ability to bind to a variety of substances, including hormones and toxins, thus demonstrating its important role in regulating physiological processes in broilers (Belinskaia *et al.*, 2021; Everaert *et al.*, 2013).

The absence of mortalities and clinical signs of problems (such as diarrhea and others), as well as the absence of weight loss in treatments T3, T4, and T5, further support this. This suggests that the addition of BSFLM enhanced production performance and had no negative effects on bird health. These results are consistent with those of Attivi *et al.*(2020) , who reported that treatment A100 had a significantly higher ($P < 0.05$) albumin concentration than the control treatment A0, A25, A50, and A75 when BSFLM was substituted for fishmeal in broiler diets. The relationship between the inclusion of BSFLM larvae in broiler diets and improved albumin concentrations and the extent to which broilers benefit from this addition can be explained as follows: BSFL larvae have a high content of amino acids, especially essential amino acids that are indispensable for the bird's body and cannot be produced internally, necessitating their acquisition from the diet (Spranghers and Vanherpe, 2019). On the other hand, albumin is rich in amino acids, particularly lysine, which is the second limiting amino acid in broiler diets (Kidd *et al.*, 2000). As a result, incorporating BSFL larvae into the broiler diet helps to increase albumin concentrations, which in turn provides a suitable and balanced source of amino acids for protein synthesis. This integration can effectively enhance growth performance, meat quality, and feed efficiency in broilers (Do *et al.*, 2021). The results of this study on albumin did not agree with those of (Pasotto *et al.*, 2020), who found no significant effect on albumin levels between the BSF-M, BSF-F, and control treatments at 27 and 37 days in a study to evaluate the immune effects of dietary treatments containing BSFL larvae in broiler quails. It is worth noting that some studies have linked the inclusion of black soldier fly larvae (BSFLM) in the diet of broilers or poultry in general with improved blood parameters by lowering triglyceride and cholesterol levels, increasing hemoglobin and packed cell values, and enhancing antioxidant status (Bongiorno *et al.*, 2022; Marco *et al.*, 2021 ; Lalev *et al.*, 2020 Gariglio *et al.*, 2019 (Dabbou *et al.*, 2018) . The results of the study did not agree with those of Raju *et al.* (2023) and Marono *et al.* (2017), who found no significant differences in albumin concentration.

AST, ALT, Uric acid, Creatinine

Data from Table 4 shows no significant differences between the experimental treatments in the concentrations of AST and ALT enzymes, uric acid, and creatinine when BSF larvae meal was added as a protein concentrate in broiler diets. AST (aspartate aminotransferase) and ALT (alanine aminotransferase) enzymes are measured to test liver function, providing valuable insights into potential liver defects or problems with protein and amino acid metabolism. The absence of deviations from the reference range, whether high or low, can serve as good indicators reflecting the health status of the birds. These results are consistent with those reported by (Bongiorno *et al.*, 2022) , (Mat *et al.*, 2022) , (Gariglio *et al.*, 2019) (Dabbou *et al.*, 2018) (Marono *et al.*, 2017) and (Schiavone *et al.*, 2017) .

The same table shows no significant differences in the concentrations of uric acid and creatinine between the experimental treatments. Uric acid is the main product of nitrogenous waste in poultry (Harr, 2002) , and its serum level reflects protein metabolism and breakdown. Creatinine, a byproduct of the breakdown of phosphocreatine in skeletal muscle, is another important indicator of protein metabolism (Piotrowska *et al.*, 2011) . Both are considered measures or indicators of kidney function, as high levels of uric acid can indicate kidney disease, while high levels of creatinine can indicate kidney damage (Karimi-Dehkordi *et al.*, 2023).

Table (3). the effect of BSF larvae powder substitution of protein concentrate in broiler feed in biochemical parameters of broiler blood serum mean ± standard error.

Treatments	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Cholesterol mg/dL	Glucose mg/dL
T1	3.8633± 0.08647	2.2800^{ab} ± .070440	1.5833± .154100	148.7800 ± 8.08259	155.3400± 9.18704
T2	3.6000± .040410	2.3967^{ab} ± .218660	1.2033± .234120	163.4800 ± 7.55427	161.8900 ± 7.34800
T3	3.9040± .058730	2.3600^{ab} ± .038080	1.5440± .051640	163.2200 ± 9.35918	173.0233 ± 5.44326
T4	4.0900± .326240	2.7033 ^{a} ± .131190	1.3867 ± 0.19768	167.5867 ± 8.76482	159.0400 ± 11.65012
T5	3.8300 ± .030550	2.1867^{b} ± .144260	1.6433± .158360	173.4200 ± 7.44867	176.7733 ± 12.25185
significant	N. S	*	N. S	N. S	N. S

N.S None Significant: * Significant (vertically different letters represent significant differences at the level of 0.05 ≥ P) T1= control treatment without additives, T2= 25% replacement of (BSF-PC) imported protein concentrate T3, = 50% replacement of (BSF-PC) replacing the imported protein concentrate, T4= 75% replacement of (BSF-PC) replacing the imported protein concentrate, T5= 100% replacement of (BSF-PC) replacing the imported protein concentrate.

In this study, the diet is largely the main factor affecting uric acid and creatinine levels in poultry. This implies that the inclusion of BSFLM larvae in broiler diets does not have any negative effects on kidney function or protein metabolism in birds. These results are consistent with those of (Bongiorno *et al.*, 2022) , (Dabbou *et al.*, 2018) , and (Schiavone *et al.*, 2017) , who reported no significant differences in uric acid or creatinine when black soldier fly larvae or their derivatives were added to broiler diets.

However, El-Kaiaty *et al.* (2022) reported a decrease in uric acid concentration in the 2% treatment compared to the 4% and 6% BSFL larvae meal treatments in broiler diets. Attivi *et al.* (2020)

observed a significant decrease in uric acid with increasing levels of replacement of fishmeal with maggot meal (0%, 25%, 50%, 75%, and 100%). The lowest concentration was recorded in the A100 treatment compared to the control treatment and the other experimental treatments.

Table (4). The effect of BSF larval powder substitution of the protein concentrate in biochemical parameters of blood serum AST, ALT, Uric acid and creatinine for broiler meat mean ± standard

Treatments	AST u/l	ALT u/l	Uric acid mg/dL	Creatinine mg/dL
T1	277.333 ± 16.73652	22.2533 ± 3.10840	3.5167± .606230	.2700 ± 0 .056860
T2	266.0000 ± 13.000	20.8200 ± 3.33818	3.6733± .587720	.1767 ± 0 .068880
T3	250.0000± 15.04438	20.4900 ± 3.45260	3.4933± .586130	.0900 ± 0 .045090
T4	258.6667± 16.59652	20.6000 ± 3.52326	3.6067± .514920	.2367 ± 0 .091350
T5	276.0000 ± 12.66228	21.7267 ± 3.16421	4.1933± .652750	.2300 ± 0 .086600
significant	N. S	N. S	N. S	N. S

N.S None Significant T1= control treatment without additives, T2= 25% replacement of (BSF-PC) imported protein concentrate T3, = 50% replacement of (BSF-PC) replacing the imported protein concentrate, T4= 75% replacement of (BSF-PC) replacing the imported protein concentrate, T5= 100% replacement of (BSF-PC) replacing the imported protein concentrate.

Humoral immune response

Table 5 shows the effect of replacing protein concentrate with BSF larvae meal in broiler diets on the volume of antibodies against Gumboro disease and immune globulins. There were significant differences ($P \geq 0.05$) in favor of treatments T3, T4, and T5, which recorded (2539.0633, 2677.5700, 2522.8133, respectively) compared to the control treatment T1, which did not differ significantly from treatment T2, which recorded (2038.8833, 2024.7900), respectively. As can be seen from Table (4), there were no significant differences in the concentrations of immune globulins IgA, IgM, and IgG between the experimental treatments. Infectious bursal disease virus (IBDV), also known as Gumboro disease, is a highly contagious viral infection that primarily affects chickens. It is characterized by a range of symptoms, the most severe of which is watery diarrhea. In severe cases, Gumboro disease can be fatal (Franciosini and Davidson, (2022) and Teshome *et al.* (2015).

BSFLM may have a positive effect on the broiler immune system against Gumboro disease. One proposed mechanism for explaining the significant improvement in the immune response of birds

in treatments T3, T4, and T5 is that BSFLM contains antimicrobial peptides (AMPs). AMPs are immune proteins that have high activity against bacteria, fungi, viruses, and parasites. AMPs can also modulate or activate the immune system by regulating the expression of inflammatory mediators, enhancing the activity of phagocytic cells, and stimulating the proliferation and differentiation of lymphocytes (Zhang *et al.*, 2022) and (Saviane *et al.*, 2021).

Table (4) The effect of BSF larval powder substitution of the protein concentrate in the volumetric norm of antibodies against Gumboro disease and broiler immunoglobulins at the age of 28 days ± standard error

Treatments	Gumboro disease	IgA mg/dL	IgM mg/dL	IgG mg/dL
T1	2038.8833[b]± 63.51311	11.1367 ± 2.08279	6.9167± 1.48958	22.0200 ± 3.55719
T2	2024.7900[b]± 69.71674	11.1633 ± 2.22063	6.7467± 1.61907	22.4767 ± 2.41303
T3	2539.0633[a]± 175.56759	13.6033 ± 1.60567	8.6867± 1.16405	31.5667 ± 4.35730
T4	2677.5700[a]± 220.75253	13.6900± 1.79552	8.0467± 1.55506	31.7900 ± 2.93333
T5	2522.8133[a] ± 24.41749	12.7733 ± 1.83396	8.0133± 1.59106	32.6400 ± 3.32580
significant	*	N. S	N. S	N. S

N.S None Significant: * Significant (vertically different letters represent significant differences at the level of $0.05 \geq P$) T1= control treatment without additives, T2= 25% replacement of (BSF-PC) imported protein concentrate T3, = 50% replacement of (BSF-PC) replacing the imported protein concentrate, T4= 75% replacement of (BSF-PC) replacing the imported protein concentrate, T5= 100% replacement of (BSF-PC) replacing the imported protein concentrate.

These results are consistent with those of de (de Souza Vilela *et al.*, 2021) , who reported that the inclusion of BSFL can improve the immune performance of broilers. The results of this study also support the findings of (Zhang *et al.*, 2021) , who investigated the effects of including black soldier fly larvae (Hermetia illucens L.) BSFL at levels of 10 and 20% on immune responses in 10-day-old chickens infected with IBV. Zhang reported that commercial feed supplemented with 10% BSFL reduced mortality (20%) and disease severity (80%), as well as IBV viral loads in the trachea (65.8%) and kidneys (20.4%) compared to the control treatment of IBV-infected chickens fed unsupplemented commercial feed. In addition, 10% BSFL supplementation resulted in an

enrichment of $CD8^+$ T lymphocytes in peripheral blood and increased interferon-gamma (IFN-γ) mRNA and protein levels in the spleen compared to the control treatment. It is important to highlight the benefits of chitin. Chitin exhibits prebiotic properties in the hindgut and appears to have an inhibitory effect on Gram-negative bacteria. Its antioxidant potential also benefits the poultry immune system. In addition, chitin has antimicrobial and antifungal properties (Elieh Ali Komi *et al.*, 2018; Borrelli *et al.*, 2017).

Lauric acid also deserves mention for its antioxidant properties and its role as a prebiotic that may improve gut immune function (Zhu *et al.*, 2020). Ensuring optimal growth performance in poultry requires utmost importance to maintaining healthy gut function and welfare. Moreover, ensuring that the poultry digestive system is healthy and functioning properly is critical to promoting optimal growth performance. There is no doubt that the immune performance of broilers can be affected by the ingredients and nutrients present in their feed (de Souza Vilela *et al.*, 2021) and (Osman *et al.*, 2010).

Conclusions

The inclusion of BSFLM in broiler diets has been shown to have a positive impact on hemoglobin levels, with no negative effects on other blood parameters or biochemical markers observed. The birds showed no signs of stress or disease. Hemoglobin levels, particularly, are important indicators of overall health and physiological well-being in broilers. These findings enhance our knowledge of sustainable and effective protein sources for poultry nutrition.

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Conflict of interest

The authors declare that there are no competing interests in the current study.

Ethical approval

This study has been approved by the General Animal Use and Care Committee, College of Veterinary Medicine, University of Basrah, Basra State, Iraq.

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Authors' contributions

TIM and QJG, –Development of the Methodology, Collection of samples, and Laboratory procedures. TIM and ASK –Preparing and writing the initial draft, review and editing the manuscript and analyze the data.

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