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Anbar Journal of Agricultural Sciences
(University of Anbar – College of Agriculture)

Effect of Supplementation of Black Soldier Fly Meal on The Performance, Blood Constitutes, And Histological Features in Fayoumi Chicks

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Article info

Received: 2025-02-18
Accepted: 2025-07-24
Published: 2025-12-31

DOI-Crossref:
10.32649/ajas.2025.189315

Cite as:
Khosht, A. R., Adbdel Latif, H., El Sabry, M. I., and Mekkawy, A. M. (2025). Effect of Supplementation of Black Soldier Fly Meal on The Performance, Blood Constitutes, And Histological Features in Fayoumi Chicks. Anbar Journal of Agricultural Sciences, 23(2): 1244-1261.

Abstract

This study investigated the influence of long-term dietary supplementation of defatted black soldier fly larvae *Hermetia illucens* meal (DBSF-M) on the live performance, carcass%, edible giblets and lymphoid organs weight %, blood biochemicals and histological features of liver and lymphoid organs in a slow-growing chicken. One hundred- eighty chicks, aged one week, were divided into three groups (group/ 5 replicates) according to the dietary DBSF-M supplementation levels. The 1st group was fed a basal diet, which served as a control. The 2nd and 3rd groups were fed basal diets with 3 and 6 g DBSF-M / kg feed, respectively, for 10 weeks of age. Then, five chicks per treatment were sacrificed to obtain organs and blood samples. Both levels of DBSF-M supplementation reduced feed intake, increased body weight gain, and enhanced the feed conversion ratio of chicks. They also increased the thymus weight and reduced liver weight. In addition, they reduced triglycerides and AST levels and increased the anti-oxidant capacity level of treated chicks. The low dose of DBSF-M (3g/kg feed) did not affect the liver, or thymus while increasing the measures of the bursa; (medulla width, cortex thickness, and follicle width), and white pulp of spleens. Meanwhile, the high dose of DBSF-M (6 g/kg feed) negatively altered the structure of the examined tissues. Conclusively, 3 g BSF/ kg feed can be a sustainable feed additive for enhancing the feed conversion ratio and immune indices of the growing chicks.

Keywords: Insect protein, Growth, Feed additive, Histomorphometry, Immunity.

فوائد ومخاطر إضافة مسحوق يرقات ذبابة الجندي الاسود منزوعة الدهن على الأداء الانتاجي، مكونات الدم والسمات النسيجية للأعضاء في كتاكيت الدجاج الفيومي

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الخلاصة

أجريت التجربة لدراسة تأثير اضافة مسحوق يرقات ذبابة الجندي الأسود منزوعة الدهن (DBSF-M) للعليقة على الأداء الانتاجي، نسبة التصافي، الوزن النسبي للأعضاء، خصائص الدم، والخصائص النسيجية للكبد والأعضاء اللمفاوية في احدى سلالة دجاج بطيئة النمو. تم استخدام عدد 180 كتكوت عمر أسبوع إلى ثلاث مجموعات (مجموعة/خمس مكررات) وفقًا لمستويات الأضافة DBSF-M. تم تغذية المجموعة الأولى على عليقة أساسية (كنترول) بدون اضافات، تم تغذية المجموعتين الثانية والثالثة على العليقة الاساسية مضافا لها 3 و6 جرام من DBSF-M / كجم من العليقة، على التوالي، لمدة 10 أسابيع من العمر. في نهاية التجربة تم ذبح خمسة طيور من كل معاملة للحصول على عينات من الأعضاء والدم. أظهرت النتائج انخفاض الغذاء المأكول في المجمعتين المغذاه على مكملات DBSF-M، ولكن زاد الوزن المكتسب، وتحسن معامل التحويل الغذائي، كما زاد وزن الغدة التيموثية وانخفض وزن الكبد. بالنسبة لمقاييس الدم: انخفضت مستويات الدهون الثلاثية وAST وزادت مضادات الأكسدة لدى الكتاكيت المعاملة. لم تؤثر الجرعة المنخفضة من DBSF-M 3 جرام/ كجم علف) على الكبد أو الغدة التيموثية، بالرغم من زيادة مقاييس البرسا (عرض النخاع، وسمك القشرة،

وعرض الحويصلة) ونسيج اللب الابيض للطحال. في حين أن الجرعة العالية من DBSF–M (6 جرام/ كجم علف) اثرت سلبًا على بنية أنسجة الأعضاء المفحوصة. الخلاصة: 3 جم من مسحوق DBSF–M بمثابة مادة مضافة مستدامة للأعلاف لتعزيز معامل التحويل الغذائي ومناعة الكتاكيت النامية.

كلمات مفتاحية: البروتين الحشري، النمو، إضافات الأعلاف، القياسات النسيجية، المناعة.

Introduction

Poultry meat is essential nourishment for humans due to its high nutritional value. However, implications of climate change such as water stress, high temperature, and avian diseases, negatively affect the health and performance of chickens (14 and 15). In this respect, suitable and alternative feed additives efficiently maintain broilers and laying hens healthy and productive flocks; various ingredients such as chitosan, nano-elements, and medicinal plants have positively impacted the performance and health of poultry species (14, 15 and 16). However, the commercial use of these products in poultry diets is hindered due to limited production, high cost, and safety issues especially nano-products (16). Thus, insect products could be applicable and eco-friendly alternative feed ingredients/ additives. For instance, it can be a source of amino acids and energy in poultry diets (1, 4 and 34).

Black soldier fly larva (*Hermetia illucens*) (BSF) meals have been employed in poultry diets as a potential solution due to their unique nutritional value such as their high content of crude protein (35-60%), and essential amino acids up to 3% lysine, 1% methionine, and 2% threonine (1, 9, 2 and 41). Moreover, the BSF nutritional profile is rich in energy (up to 42% fats), fatty acids such as lauric acid, and crucial minerals such as calcium and phosphorus (30). From a safety perspective, BSF-processed meals are pathogenic-agents-free (11, 12 and 13). A small production unit of BSFL can be established by small stakeholders and organic meat producers to provide natural supplementation for their flocks.

The BSFL meal has been successfully utilized in chicken broiler diets as a partial substation for soybean and corn to enhance performance, carcass, gut microbiome, and meat quality parameters (4 and 7). For example, (5) showed that dietary inclusion of 16% BSFL meal as a complete replacement of SBM reduced dietary available energy and nutrient digestibility, leading to reduced growth performance of broiler chickens. These effects may have been associated with high dietary chitin content and inaccuracies in dietary formulation assumptions regarding NPN and fat digestibility values. However, feeding BSFL reduced excreta moisture content, suggesting that BSFL meal may be beneficial in improving litter quality. On the other hand, (, 40 and 41) found positive growth performance of chickens fed 5% BSFL. Also, (17 and 18) indicated that lower levels of BSFLM could provide some growth-promoting effects commensurate to antibiotics in the starter phase. However, replacing soybean meal with greater amounts (50 %) of BSFLM reduced growth and increased organ size.

In Egypt, the majority of local chicken breeds, Fayoumi chickens have a high immunity level and are well adapted to harsh conditions with acceptable production performance at the level of a small-scale production system.

Although previous studies showed the feeding value of the BSF meal in meat- and egg-type chickens, there is a need to investigate the effects of the dietary supplementation of BSF on slow-growing chicks, and if there are negative effects due to different dietary BSF levels supplementation on the animal well-being. Therefore, the present study aimed to investigate the effects of two levels of dietary DBSF-M supplementation on the performance, blood biochemistry, and histological features in slow-growing chicks.

Materials and Methods

This study was conducted in the El Fayoum Poultry Research Station, Animal Production Research Institute, Agricultural Research Center, Egypt. The experimental design was approved by Animal Care and Use Committee protocol number (VET-CU-13102024956).

Defatted Black Soldier Fly (*Hermetia illucens*) larvae meal (DBSF_M) was obtained from the RandD Department of EGYMAG®, Qalubia, Egypt. The conditions for developing BSF were grown under optimal conditions and fed vegetable wastes (10).

Defatted BSF meal (DBSF-M) was processed as follows: 1) larvae were collected in their final instar, with an average weight ranging between 180 and 230 mg / larva. Then, the larvae were dried for 20 hours at 60 °C before being processed into a meal. The BSF meal was defatted using high pressure without employing any solvents. Finally, DBSF-M was analyzed to determine 1) the chemical composition and energy value of DBSF-M ingredients according to AOAC procedures (2). 2) amino acid profile using high-performance liquid chromatography (HPLC) with column: LCAKO6Na, 570nm - 440nm.

Birds and experimental design: In December 2023, a total of 180 one-week-old Fayoumi chicks (Takamoli Station, Fayoum, Egypt) were employed in this experiment. Chicks were raised in pens equipped with standard drinkers and feeders separated by a wooden barrier, in an open-sided house. The ambient temperature was 32 C◦ during brooding time (1^{st} week) and ranged from 28 to 24 C◦ until the end of experimental time. The lighting regimen of 22 hours of light and 2 hours of darkness, during the first day, then from day two until the end of the experiment, a lighting regimen of 16 hours of light followed by 8 hours of darkness/day was applied. Feed and water were available *ad libitum*.

From day one of the experiment, the chicks were randomly assigned into three dietary treatment groups (5 replicates of 12 chicks each) according to the inclusion level of DBSF meal. The first group of birds was fed a basal starter (1 day–6 weeks of age) and grower diets (7–10 weeks of age) and served as a control group (0% BSFL meal). The 2nd and 3rd groups of chicks were fed basal starter and grower diets with 3 and 6 g DBSF-M/ kg diets, respectively (Table 2). The nutritional values of the formulated diets were determined according to the AOAC guidelines (2).

Growth performance parameters: During the experimental period (10 weeks), body weight (BW), Feed intake (FI), and mortality were recorded daily. Then, BW, FI, live body weight gain (LBWG), and feed conversion ratio (FCR) values were calculated and presented for the periods from 0 - 6, 7-10, and 0-10 weeks.

Organs and blood sampling: At the 10th week of age, 15 blood samples (5 samples/dietary group) were collected in serum-separating tubes without anticoagulant. Subsequently, the samples were centrifuged at 3000 rpm for 10 minutes to collect serum that stored at -20^{o}C.

The triglyceride, cholesterol, aspartate aminotransferase (AST), creatinine, and urea concentrations were measured using specific kits following the manufacturer's recommendations, using a digital biochemical analyzer. The total antioxidant capacity (TAC) was analyzed using commercial kits (Randox, UK).

Also, five birds per dietary treatment were chosen based on the average body weight of each treatment and sacrificed to measure carcass yield, giblets (liver, gizzard, and heart), and lymphoid organs (bursa, thymus, and spleen) weights, then the relative weight of organs was calculated as percentages of live body weight.

Histopathological investigations: After slaughtering, spleen, liver, thymus, and bursa of Fabricius samples were collected and immediately fixed in a 10% buffered formalin solution, embedded in paraffin wax blocks, sectioned at a thickness of 5 µm, mounted onto glass slides. Consequently, they were stained with Hematoxylin and Eosin (HandE) stain for the histopathological examination under light microscopy (3 and). The histo-morphological descriptions of the bursa *Fabricius*, including the cortex thickness, medulla area, and width of the follicles were done according to (34).

Statistical analysis: Data were analyzed using the SPSS, 2007 package (45). One-way analysis of variance (ANOVA) was carried out to explore the effect of the DBSF-M level on all the growth performance and carcass traits. Duncan's test considers the level of statistical significance at ≤5%.

Table 1: Ingredients and chemical composition of diets.

Ingredients (g/kg as fed)	Starter diet (1-6 Weeks) dietweek	Grower diet (7-10 Weeks)
Yellow corn	63.5	70
Soybean meal	32.5	26
Di-calcium phosphate	2	2
Limestone	1	1
Salt (NaCl)	0.45	0.45
Minerals*	0.3	0.3
Vitamins**	0.1	0.1
Methionine	0.15	0.15
Total	**100**	**100**
Calculated nutrients ***		
Crude protein %	19.7	17
Met. Energy (kcal/kg)	2798	2906
Calcium%	0.93	0.91
Available Phosphorus %	0.51	0.5
Lysine %	1.04	0.88
Methionine	0.46	0.44
Methionine+cysteine	0.79	0.65

*Supplied minerals per kg of diet: Copper 4 mg; Iron 30 mg; Manganese 60 mg; Zinc 50 mg; Iodine 0.3 mg; Cobalt 0.1 mg and Selenium 0.1 mg.

**Supplied vitamins per kg of diet: Vit. A, 10000 IU; Vit. D3, 2000 IU; Vit. E, 10 mg; Vit K3, 1 mg; Vit. B1, 1 mg; Vit. B2, 5 mg; B6, 1.5 mg; B12, 10 mcg; Nicotinic acid 30 mg; Folic acid 1mg; Pantothenic acid 10 mg and Biotine 50 mcg; Choline 250 mg.

*** According to the Egyptian Regional Center for Food and Feed (RCFF, 2001).

Results and Discussion

Amino acids profile: Results in Table 2 showed that DBSF-M contents of CP (55%) and energy (2800 kcal/ kg) are high, which indicates the potentiality of the BSF-M as a source of protein and energy in diets. Similar energy values were reported by (22), but the amino acid profile of the DBSF-M was different. Compared to the values of the amino acids by (22), the aspartic acid, methionine, threonine, glycine, alanine, cysteine, and isoleucine values were higher (Table 2 and Fig.1).

Table 2: Composition and amino acids profile of DBSF-M.

Item	Amount
Crude protein	55%
Met. Energy	2799 kcal/kg
Calcium	2.5%
Phosphorus	1%
Amino Acid	%
Aspartic Acid	10.1
Threonine	2.6
Serine	2.4
Glutamic Acid	14.9
Glycine	5.5
Alanine	6.8
Cysteine	8.7
Valine	1.1
Methionine	4.1
Isoleucine	8.0
Leucine	3.9
Tyrosine	4.1
Phenylalanine	7.9
Histidine	7.8
Lysine	11.2
Ammonia	**0.8**

It is noteworthy that the BSF content of CP, fat, and fiber % could vary due to different BSFL rearing systems *e.g.* (28, 43 and 44) and (, 20 and 33) found that the CP, fat, and fiber content of BSF-M is 44.9, 29.1, and 16.4 %, respectively. Moreover, the amino acid content of DBSF-M is better than that of plant protein sources such as soybean (23, 29). Also, (8 and 34) indicated that the overall results indicated that the BSF-M and DBS-M contain approximately 0.8% digestible P. They suggested that compared to plant feedstuff e.g. corn and soybean, higher relative bioavailability of P in BSF-M is due to a lack of phytate in BSFL. Therefore, BSFL can be suggested as a substantial source of available P in poultry diets.

Using smart systems can be an efficient tool for governing the BSF factor operation, which can increase the quality of products, and may lead to better use of the products such as determining the optimal dosage based on accurate information.

Growth performance: At week 6 of age, dietary DBSF-M supplementation did not affect the LBW of chicks (P > 0.05). At the 10th week of age, the LBW of chicks who received dietary DBSF-M supplementation was greater than that of chicks of the control group; however, only the LBW of chicks that received a 3 g DBSF-M /kg feed was significantly greater than this of chicks in the control group. It can be suggested that the effect of DBSF-M supplementation became notable when the chick's diet was changed from a starter (high CP %) to a grower diet (lower CP %). From, a commercial perspective, these results could show that the use of BSF meals as a feed additive could be more efficient than using BSF as a substitute for soybean. In this regard, (17 and 25) mentioned that the replacement of 50% and 100% of soybean with BSF meal significantly increased the BW of chicks; but from an economic perspective, 50% replacement compromised neither commercial performance.

Also, the dietary DBSF-M inclusion did not improve the BWG during the first half of the experiment (0-6 weeks). While, from 7 to 10 wk of the experimental period, the chicks' BWG of both 3 and 6g DBSF-M groups were greater compared to the BWG of chicks of the control ones. However, only the chicks that received the 3 g DBSF-M/ kg diet supplementation had significantly greater BWG than the BWG of chicks that received basal diet (Table 3). From 0-10 weeks of the experiment, the overall BWG of chicks that received dietary 3 and 6 g DBSF-M supplementation were numerically higher compared to the BWG of chicks that fed on the control diet, which could refer to a growth-promoting effect of the DBSF-M.

Along the same line, the dietary DBSF-M inclusion did not affect the FI and FRC during the first six weeks of the experimental period. But, from 7 to 10 weeks and overall the experimental period (0-10 weeks), both levels of DBSF-supplementation significantly reduced the FI of chicks compared to the control group. Also, it is worth noting that the FI of the 3 g DBSF-M group was significantly higher than that of the 6g DBSF-M group. Only during the period from 7-10 weeks of the experimental period, the FRC of both DBSF-M groups were better than that of the control group (Table 3). This could be due to the enhancement of the gut health and intestinal microbiome. The antimicrobial compound in *H. illucens* larval extract affects the activity of several G-positive and G-negative microorganisms (4 and 7). Amongst these effective antimicrobial compounds is antimicrobial proteins such as defensins, cecropins, attacins, and diptericins. Also, Chitin, which can be found in the exoskeleton of the larva, is also associated with the defense mechanisms against some parasitic infections (26 and 42). This may enhance the gut microbiome that reflect on the intestine functions and finally enhance the BWG and FCR.

From circular and environmental aspects, BSFL is identified as the most versatile in terms of the variety of bio-waste (34). Therefore, bringing the BSF-M into compliance with the principles of the circular economy increases its competitiveness by reducing production costs and improving productivity. It is worthy to mention that all the chicks stayed healthy during the experiment and did not have symptoms associated with any disease.

Table 3: Effects of different levels of DBSF-M on the body weight, body weight gain, feed intake, and feed conversion ratio.

Items (g)	Age	Dietary treatments			SEM±	P-value
		Control	DBSF 3 g	DBSF 6 g		
LBW	Initial Wight	57.9	56.7	57.3	0.34	0.39
	6 wks	457	455	447	3.7	0.53
	10 wks	890b	930 a	910 ab	7.5	0.01
BWG	0 - 6 wks	401	405	393	3.3	0.33
	7-10 wks	449 b	482 a	470ab	5.1	0.02
	0-10 wks	844	880	857	7.2	0.10
FI	0 - 6 wks	1317	1315	1317	1.0	0.12
	7-10 wks	1451 a	1443b	1425 c	1.1	0.01
	0 - 10 wks	2769 a	2758 b	2742 c	1.1	0.01
FCR, g/g	0 - 6 wks	3.33	3.28	3.38	0.03	0.31
	7-10 wks	3.28 b	3.06 a	3.08 a	0.03	0.01
	0-10 wks	3.32	3.17	3.23	0.03	0.08

DBSF 3 g: a basal diet with 3 g defatted black soldier fly meal /Kg feed, DBSF 6 g: a basal diet with 6 g defatted black soldier fly meal /Kg feed, FI: feed intake, FCR: feed conversion ratio, LBW: live body weight, BWG: body weight gain, SEM: standard error of the mean, the means within the row, followed by different superscripts are significantly different ($p < 0.05$).

Carcass characteristics: In Table 4, the current study found that supplementing with 3 or 6 g DBSF-M did not affect the carcass and abdominal fat relative weights at the 10th week of age. Similarly, BSF meal did not affect the meat quality and carcass yield % of meat-type chicken (7 and 44). Also, it was found that the replacement of 50% soybean with BSF meal enhanced growth performances, but it did not influence carcass weight (25). In addition, they reported that the inclusion of BSF-M in the diet increased the saturated fatty acids and negatively affected breast meat n6 /n3 ratio. According to (19 and 39), the improper saturated fatty acid / polyunsaturated fatty acids ratio means a decline in the meat quality.

In addition, gizzard, and heart weights relative weights were not affected by DBSF-M supplementation, but the liver relative weight of the chicks in the control group was the highest compared to those of the DBSF-M groups ($p = 0.003$). Nevertheless, the liver relative weight of chicks who received DBSF-M supplementation/ kg feed is within the acceptable range (Table 4).

Regarding immune organs, the thymus relative weight of chicks received a 3 g DBSF-M/kg feed was the heaviest among those of the chicks of other groups. While the relative weights of the spleen and bursa (%) were not significantly affected by the tested levels of DBSF-M supplementation. In the same line, the partial inclusion of DBSF-M affected the histological features of the spleen in Muscovy duck (19). Also, the functional activity of the spleen is highly related to the genetic background of chicks (42). Therefore, it could be suggested that high variability in spleen weight could be expected due to different progenitors, especially in indigenous ecotypes.

Table 4: Effects of different levels of DBSF-M on carcass characteristics of Fayoumi chicks.

Items (%)	Dietary treatments			SEM±	P-value
	Control	BSF 3g	BSF 6g		
Carcass yield	65.47	64.51	63.76	0.35	0.13
Abdominal fat	0.343	0.364	0.582	0.05	0.17
Liver	2.78 a	2.31 b	2.37 b	0.07	0.003
Gizzard	1.763	1.804	1.838	0.04	0.76
Heart	0.617	0.577	0.582	0.01	0.27
Immune organs					
Spleen	0.38	0.35	0.33	0.02	0.17
Thymus	0.41b	0.51 a	0.37b	0.03	0.04
Bursa	0.33	0.27	0.31	0.04	0.06

DBSF 3 g: basal diet with 3 g defatted black soldier fly meal /Kg feed, DBSF 6 g: basal diet with 6 g defatted black soldier fly meal /Kg feed, SEM: standard error of the mean, the means within the row, followed by different superscripts are significantly different ($p < 0.05$).

Blood Parameters: The literature showed that there are discrepancies in the results of blood profiles, which are due to strain, age, and environmental conditions (12, 13 and 20). However, the obtained blood parameter values generally are within the specified range for growing chickens (13 and 19). Table 5 indicates the influence of feed supplementation with 3 and 6 g DBSF-M / kg feed on the blood biochemical at 10 wk of age.

Regardless of the DBSF-M dose, the blood cholesterol level of chicks in the control group was the lowest compared to treatment groups. On the other hand, chicks of the treated groups had significantly lower values of triglyceride, AST, creatinine, and urea compared to those of chicks in the control group. In comparison, dietary DBSF-M supplementation did not affect ALT levels (Table 5). In this context, high levels of triglyceride, ALT, AST, and creatinine could be a physiological stress response in chickens (19 and 26). Similarly triglyceride, AST, and creatinine levels were reported when they utilized 5, 10, and 15% of DBSF-M ere used as a protein source in the diet (6). Moreover, both levels of dietary DBSF-M significantly increased the TAC values compared to those of chicks in the control group (Table 5). Thus, it can be supposed that these blood parameters levels may refer to the improvement in the health and well-being of chicks.

Chitin is a nitrogen-containing polysaccharide found in the cuticle of *H. illucens* larvae (10). Chitin fibers can be used as absorbable sutures and for wound treatment, as they are capable of accelerating the wound healing process (27 and 33). Chitin also increases the weight of lymphoid organs and enhances both humoral and adaptive immunity by recruiting and activating immune cells. Additionally, chitin and its derivatives possess scavenging activity that helps eliminate free radicals. Its antioxidant properties can protect the body from damage caused by oxidative stress (27, 35 and 40). These findings support the current results regarding thymus weight and antioxidant capacity.

On the other hand, the present TAC result is in contrast with the findings of (7), who showed a linear response of glutathione peroxidase (GPx) activity (as an indicator of antioxidant capacity) to the increase of DBSF-M level. This result may be due to higher levels of the BSF-M.

Table 5: Effect of defatted dietary black soldier fly (BSF) meal on serum constituents of Fayoumi chicks.

Items	Dietary treatments			SEM±	P-value
	Control	BSF 3g	BSF 6g		
Triglyceride mg/dL	100.3[a]	73.8[b]	77.14[b]	4.3	0.001
Cholesterol mg/dL	153.7[b]	179.7[a]	175.6[a]	3.7	0.004
AST U/mL	66.4[a]	51.7[b]	45.0[b]	3.1	0.008
ALT U/mL	29.90	32.50	31.40	1.1	0.652
Creatinine g /dL	0.83[a]	0.68[b]	0.69[b]	0.05	0.001
Urea mg/dL	32.62[a]	26.93[b]	28.67[b]	0.77	0.002
Total Antioxidant Capacity. mM/L	0.75[b]	1.03 [a]	0.96[a]	0.04	0.001

ALT: alanine transaminase,AST: aspartate aminotransferase, DBSF 3 g: basal diet with 3 g defatted black soldier fly meal /Kg feed, DBSF 6 g: basal diet with 6 g defatted black soldier fly meal /Kg feed, SEM: standard error of the mean, This means, that within the row, followed by different superscripts are significantly different ($p< 0.05$).

Histopathological and Histomorphological Findings: The liver tissue architecture of the control group chicks (G-I), and the 3g DBSF-M group (G-II) were normal (Fig. 2 A and B). Mild multifocal lymphoplasmacytic inflammatory infiltrates with or without Fibrous tissue deposition, and mild vacuolar degeneration of the hepatocytes were observed in the 6g DBSF-M group (G-III) (Fig. 2C). The histopathological investigation showed that the dietary 3g DBSF-M/kg feed had no adverse effect on liver tissue, whereas the 6g DBSF-M/kg feed exhibited a mild effect. These findings suggest a low-grade inflammatory immune reaction to dietary components or metabolic stress (6). The presence of lymphocytes and plasma cells suggests a chronic inflammatory process, possibly related to antigenic stimulation or altered gut-liver axis signaling resulting from the inclusion of insect-derived proteins in the diet. Additionally, the mild vacuolar degeneration observed in hepatocytes typically reflects early cellular injury, often linked to metabolic disturbances such as lipid accumulation or mild oxidative stress (34). This agrees with the findings of (46). Similarly, in Japanese sea bass-fed diets, including defatted BSF prepupae meal led to minor effects on the liver. Also, (7) reported no significant alterations in the liver of broilers fed on diets supplemented with DBSF L meal. Based on these findings, the 3g DBSF-M treatment did not affect the birds' health.

Immune organs: The bursa medulla of G-II chicks (325773 μm^2) was larger than the medulla of the bursa of Fabricius of the G-I (142323 μm^2) and G-III (307253 μm^2) chicks (Fig. 2D-F). Also, the bursa of the bursa of *Fabricius* cortex of chicks of the G-II (138.13 µm) was thicker than those of the G-I (114.63 µm) and G-III (126.14 µm) groups. The width of the bursa follicles of the chicks of G-II (852.53 µm) was wider than those of the G-I (420.15 µm) and G-III (631.39 µm), which refers to more lymphocyte cell production in the 3 g DBSF-M treatment compared to other treatments. Also, (31) found that adding combination of Indigofera zolliengeriana powder up to 10% and 25% BSF-M to the starter diet increased the medulla width and cortex thickness of chickens. The bursa of *Fabricius* has follicles consisting of a cortex and a medulla. It is known that most cell division occurs in the cortex, which consists of lymphocytes, plasma cells, and macrophages (24). The medulla only contains lymphocyte cells, which function to produce antibodies in chickens (12 and 21). In the G-II group, a larger medulla area and more lymphocyte cells existed compared to those of other groups. In addition, it was reported that the width of the follicle was the greatest in 3g DBSF-M treatment, which indicates an increase in the number of B lymphocytes for antibody production. The supposed high number of B lymphocytes in the 3 g and 6g DBSF-M treatments could be due to the DBSF-M content of immune-modulatory substances. For instance, larvae BSF has high lauric acid content and can function as a natural antimicrobial agent (2). Moreover, this result could be supported by the enhancement of the antioxidant capacity of the treated birds as shown in the blood parameter section of this study.

Histopathological examination of the spleen from the control group showed a normal histological structure (Fig. 3A). The spleen of chicks from the G-II showed mild multifocal white pulp hyperplasia (Fig. 3B). This may indicate a better capacity for antibody production (15). While the spleen from the G-III revealed marked vasculitis (Fig. 3C). Also, a microscopical picture of the thymus obtained from the G-I and G-II showed normal histological architecture (Fig. 3D and E). On the contrary, a mild multifocal cortical depletion was observed in the thymus from the G-III (Fig. 3F). These results are contrary to (7), who did not find any significant effect on the severity of the histopathological alterations in broiler chickens fed diets with BSF inclusions. Therefore, the varying degrees of lymphoid system activation observed in the chicks of the present study could be related to various stressful conditions that frequently occur in some poultry-rearing systems (31 and 42).

Thus, a solution to enhance chickens' immunity in is to provide functional additives such as low doses of BSF with. This is due to the content of benifical compounds that can improve the growth of the immune organs (27 and 48). While high doses of the BSF may adversely affect chicks' performances.

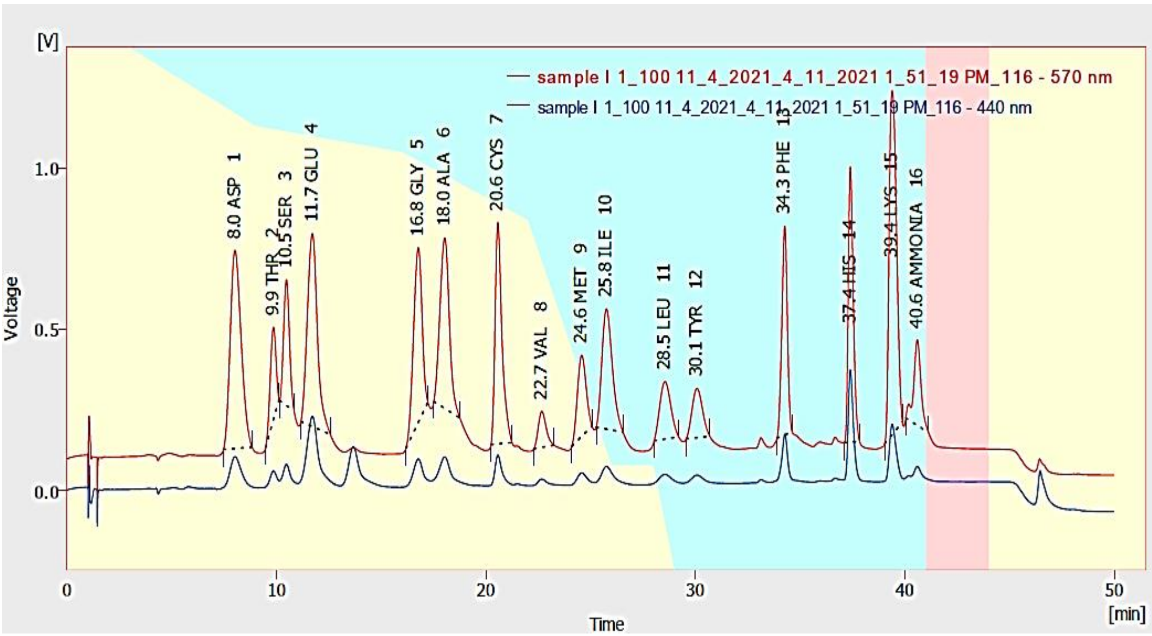

Fig. 1: Amino acids profile of DBSF-M using HPLC.

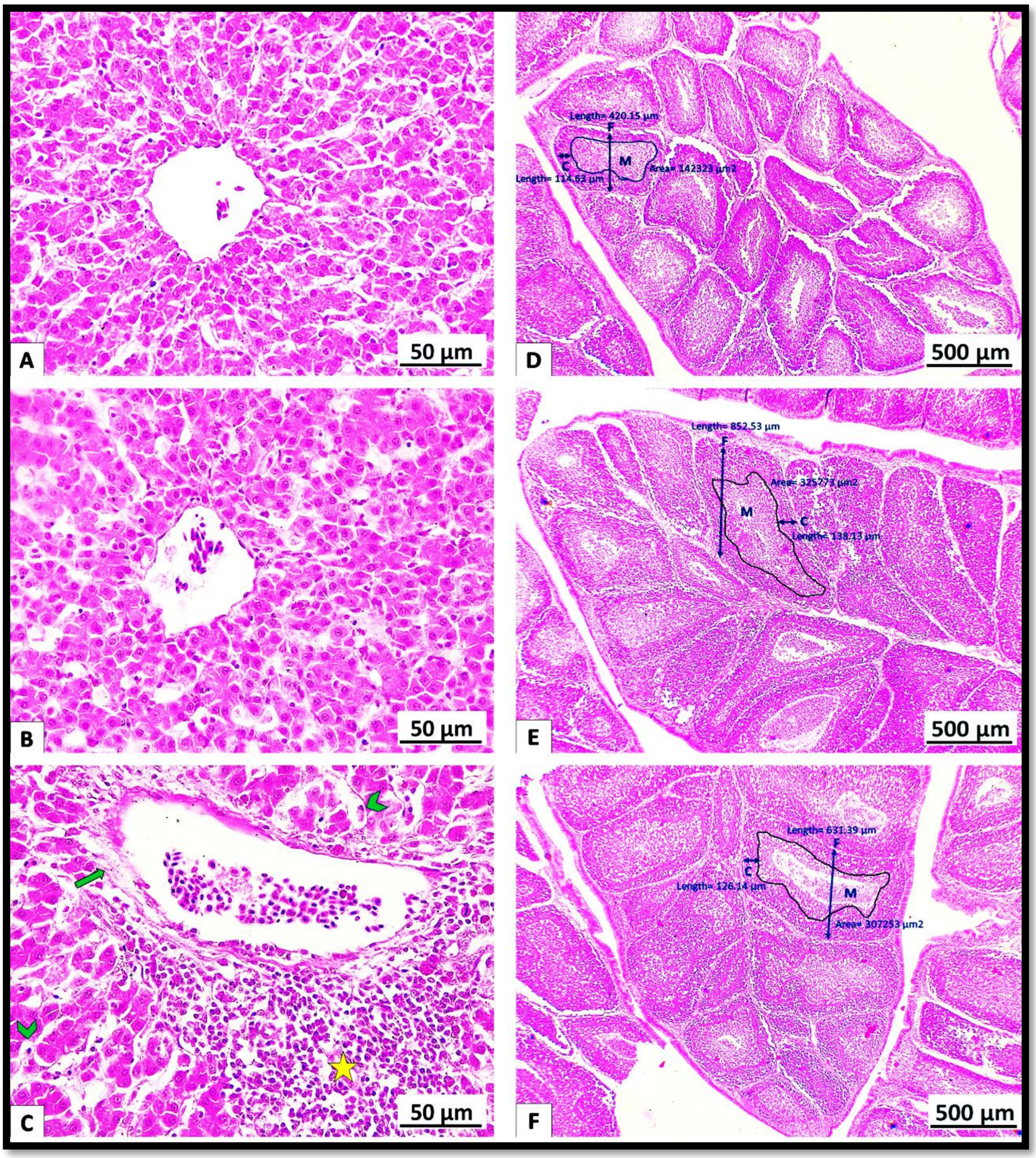

Fig. 2: Photomicrograph of (A-C) liver and (D-F) bursa of Fabricius tissue sections of chicks stained with HandE representing: (A-C) Liver from: A. Control group I, B. 3g DBSF-M group II showing a normal histological structure of the hepatic parenchyma and C. 6g DBSF-M group III showing mild multifocal lymphoplasmacytic inflammatory infiltrates (star) with Fibrous tissue deposition (arrow) as well as mild vacuolar degeneration of the hepatocytes (arrowheads). HandE, X400. and (D-F) Bursa of Fabricius from: D. Control group I, E. 3g DBSF-M group II, and F. 6g DBSF-M treatment group III: showing that the medulla in the 3g DBSF-M group was wider than in the control and 6g DBSF-M groups, the cortex in the 3g DBSF-M group was thicker than the control and 6g DBSF-M groups and the width of the bursa follicles in the 3g DBSF-M group was wider than those of the control and 6g DBSF-M groups. HandE, X40. Abbreviations: F. follicles, C. cortex and M. medulla.

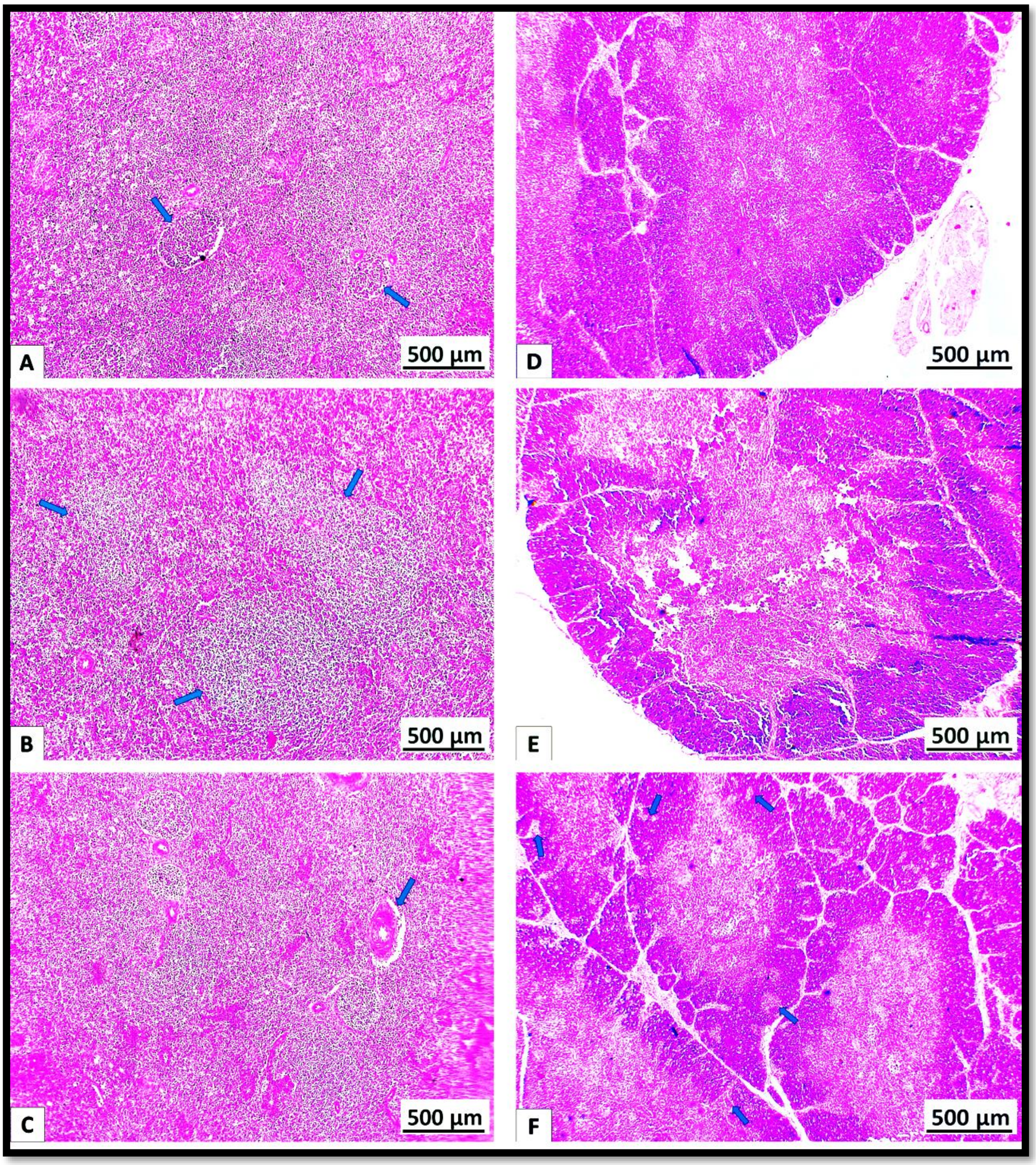

Fig. 3: Photomicrograph of (A-C) spleen and (D-F) thymus tissue sections of chicks stained with HandE representing: (A-C) Spleen from: A. Control group I showing a normal histological structure of the spleen white pulp (arrow) B. 3g DBSF-M group II showing mild, multifocal white pulp hyperplasia (arrows), and C. 6g DBSF-M group III showing marked vasculitis (arrow). and (D-F) Thymus from: D. Control group I, E. 3g DBSF-M group II showing normal histological architecture, and F. 6g DBSF-M group III showing mild, multifocal cortical depletion (arrows). HandE, X40.

Conclusions

In conclusion, using a 3g of DBSF-M per kg total feed could enhance the feed conversion ratio of chicks. Also, it maintains the structure of liver and lymphoid organs. Finally, organic poultry breeders may be encouraged to use this product to increase their flocks revenue.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Mohamed I. El Sabry; Abeer R. Khosht and Hesham AbdElatif: designing and conducting the experiment and writing the original manuscript. Aya M. Mekkawy: conducting histopathological and investigations writing of the original manuscript and revising the final version.

Funding:

This research received no external funding.

Institutional Review Board Statement:

Experimental procedures were approved by the Institutional Animal Care and Use Committee in the Faculty of Veterinary Medicine, Cairo University, Egypt, with a number (VET-CU-13102024956).

Informed Consent Statement:

Not applicable.

Data Availability Statement:

Data available upon request.

Conflicts of Interest:

The authors declare no competing financial or non-financial interest.

Acknowledgments:

The authors are thankful for the help of the RandD Department of EGYMAG®, Qalubia, Egypt for providing defatted BSF-meal.

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