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The Impact of Black Soldier Fly (*Hermetia illucens*) as Feed Supplementation on Productive and Physiological Performance of Broiler Chickens

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ABSTRACT

A total of 450 broiler chicks (Ross 308) were used to evaluate the effect of different inclusion levels of a partially black soldier fly (BSF), BSF Powder (BSFP), BSF Puré (BSFPr), and BSF frozen whole larvae (BSFL) on the growth performance, blood parameters, humoral immune response, and intestinal bacterial count of broiler chickens. The chickens were reared from day 1 to 35 and assigned to the control and 9 dietary groups with different forms of BSF (3 replicates per group with 15 chicks). Black soldier fly was included at levels of 2%, 4%, and 6% for BSFP, BSFPr, and BSFL, respectively, in the starter and growing diets. The results indicated similar body weight, weight gain, and the growth rate in chickens fed 4% BSFP, and 2% BSFPr during the experiment. There was a marked difference in blood parameters due to the different BSF forms and included percentages. The humoral immunity antibody titers against the Newcastle disease virus fluctuated among the experimental groups of different ages. Finally, it could be concluded that the BSF can be incorporated at a level of 4% in the form of powder and Puré in a broiler diet which seemed to be adequate to achieve the favorable results in growth performance, blood parameters, immunity, and bacteriological examination.

Keywords: Black soldier fly, Insects, Black solidier fly powder, Humoral immune response, Soybean substitution

INTRODUCTION

The world population growth has led to a food shortage, water wars, climate change, and soil erosion. Accordingly, the United Nations launched the sustainability initiative with 17 goals some of which are no hunger, zero poverty, good health, and well-being, and climate action (Viana et al., 2022). In this regard, all countries sought to reinforce sustainability in all life aspects, which places more pressure to find a creative solution to increase protein production so that, insects are expected to be human beings' food by 2035 (UNDRR, 2015). For hundreds of years, mankind has tried to combat insects by spending billions of dollars to eradicate insects in order to protect their crops. To extract a small amount of plant protein, they are used to killing a rich source of protein. As insects contain up to 75% protein wherever crops contain up to 14% protein (Premalatha et al., 2011).

Generally, humans hate eating insects, which prevents them from using insects as feed. However, residents of some countries have eaten insects for thousands of years, and this practice is called entomophagy. Black soldier fly is used as a partial substitution for soybean meal in commercial diets and it is a cheap replacer because they can be raised on plant or food byproducts (Schiavone et al., 2017a). Black soldier fly larvae (BSFL) are rich in essential amino acids, such as lysine and methionine, which are important for poultry production (Spranghers et al., 2017). There are more than 470 edible insect species in Africa (Ayieko et al., 2010). As a result, people can benefit from insects by using them in poultry feed (Spranghers et al., 2017).

Poultry production represents a huge sector in the animal production scale and it is of significant importance to pay close attention to poultry nutrition since 70% of the production cost is for. The soybean is going expensive day by day, and this increases the poultry production cost besides reducing the revenue of the invested pound Van (2003).

Black soldier flies have also been observed to lessen the mass and nutrient content of pig manure with similar efficiency to those of poultry manure (Li et al., 2011; Zhou et al., 2013). The meat quality of chickens fed BSF has been similar to that of the chickens fed commercial diet, therefore, they can be used as a partial substitution for soybean meal (Pieterse et al., 2019). As a result, it is well documented that BSFL may be used to feed a wide range of vertebrates (Tomberlin et al., 2015). Black soldier fly larvae also utilize a variety of vertebrate byproducts as a substrate, with no

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adverse impacts on the quality of meats grown on BSFL for humans and solution for sustainable and economic agriculture in developing countries (Diener et al., 2011; Nyakeri et al., 2017).

Black soldier fly larvae could be used as a partial replacement for chicken feed by providing additional protein in addition to being raised on chicken excrement. With this in mind, the present study aimed to investigate the effect of different forms of BSF (*Hermetia illucens*) on broiler production performance, physiological parameters, immune responses, and bacteriological examinations.

MATERIALS AND METHODS

Ethical approval

All samples were taken according to the standard protocol without causing any discomfort or injury to the chickens and the study was carried out according to the Institutional Animal Care and Use Committee of Cairo University (CU-IACUC) Medical Veterinary and Agricultural Sciences board, Egypt under approval code CU/II/F/18/21.

Study design

A total of 450 broiler chicks (Ross 308) aged one day with an average weight of 42 ± 1 g were used for the current study. The chicks were wing-banded, and then divided randomly into 9 experimental and control groups (45 chicks each) according to the BSF form (EgyMag company, Egypt), each group had 3 replicates (15 chickens per each) using a completely randomized design. Chicks from 9 groups were fed a partially replaced-soybean meal diet with different forms of BSF, including BSFP, BSFpr, and BSFL. The first three groups received BSFP at levels of 2%, 4%, and 6%. Groups 4-6 received BSFpr and groups 7- 9 gained BSFL at the same levels of 2%, 4%, and 6%.

Chicks were kept in cages (1 m in length, 0.6 m in width, and 0.4 m in height) under similar, standard hygienic, and environmental conditions. Chicks were vaccinated using Hitchner B1 strain, H5N1, and Gumboro vaccines at 6, 10, and 14 days of age, respectively. The samples were revaccinated against Newcastle disease virus (NDV) at 20 days of age according to CEVA animal health company, France (Newcastle, Gumboro, and Influenza). Batteries brooders with electric heaters were used for brooding chicks. The brooding temperature was maintained at 35°C for the first five days, then decreased by 2°C weekly until the end of week five. The lighting program was a 24-hour light for the first five days, then decreased to 22 hours from day 6 to 35. Chicks were fed starter (1- 14 days) and grower (15-35 days) diets as shown in Table 1. Feed and water were offered *ad libitum* (Okasha, 2021).

Table 1. Composition and chemical analyses of starter and grower diets of broiler chickens

Ingredients (%)	Starter (1-14 days)	Grower (15-35 days)
Yellow corn	56	59.89
Soybean meal (46% protein)	32	28.42
Corn gluten	6.05	4.95
Soya oil	1.5	2.53
Mono-calcium phosphate	1.55	1.38
Limestone	1.75	1.7
Premix (Vitamin+Mineral)*	0.2	0.2
D.L. Methionine	0.22	0.22
L. Lysine Hcl	0.25	0.25
Salt	0.40	0.40
Chemical analyses		
Choline Chloride	0.06	0.06
Crude protein	23	21
Metabolizable energy (kcal/kg)	3000	3100
Calcium	1.0	0.94
Available phosphorus	0.49	0.44
Lysine	1.4	1.3
Methionine	0.67	0.61
Methionine + Cystine	1.04	0.95
Sodium	0.18	1.8
Total	100	100

*Each 2 grams of premix mixture contained Vitamin A (trans-retinyl acetate, 9000 IU), Vitamin D3 (cholecalciferol, 2600 IU), Vitamin E (dl-α-tocopherol acetate, 16 mg), Vitamin B1 (1.6 mg), Vitamin B2 (6.5 mg), vitamin B6 (2.2 mg), Vitamin B12 (cyanocobalamin, 0.015 mg), Vitamin K3 (2.5mg), choline (choline chloride, 300 mg), nicotinic acid (30 mg), pantothenic acid (d-calcium pantothenate, 10 mg), folic acid (0.6 mg), d-biotin (0.07 mg), manganese (MnO, 70 mg), zinc (ZnO, 60 mg), iron (FeSO4 H2O, 40 mg), copper (CuSO4 5H2O, 7 mg), iodine ([Ca(IO3)2], 0.7 mg) selenium (Na2SeO3, 0.3 mg)

Estimated parameters and data collection

Production performance

Each chicken was weighed biweekly. Weight gain and growth rate were calculated according to Broody (1949).

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Blood plasma constituents

Three heparinized blood samples from each treatment were drowned randomly from the wing vein for chemical analyses at the end of the experiment. The chemical analyses were carried out by colorimetric method using a commercial kit (Biodiagnostic company, Egypt) for determining plasma Protein (total protein and albumin), lipid profile (triglycerides, total cholesterol, low-density lipoproteins (LDL), and high-density lipoproteins (HDL), and Kidney function (uric acid).

Humoral immune response

The coagulation test was used to determine antibody titer against the Newcastle disease virus following Swayne's method (1998). The serum samples were randomly selected from the wing veins of chicks in each treatment at 18, 23, and 28 days of age. Serum samples were subjected to the hemagglutination inhibition assays using a method described by Oberländer et al. (2020). The geometric means of serum hemagglutination inhibition titers obtained from each group were defined as the reciprocal logarithm in a base of 2 of the highest serum dilutions completely inhibiting agglutination.

Intestinal bacteriological count

Bacteriological examinations were carried out at 3 and 5 weeks of age using standard methods for aerobic bacteria (Brown and Smith, 2014). Microbiological analyses were detected at the department of microbiology, Cairo University, Egypt. Under complete aseptic conditions, 5 grams of broiler intestine samples with a length of 2 cm were separated and cut-opened before weighted and transferred into sterile 50 ml Falcon tubes containing 30 ml of sterile saline solution (0.85% NaCl). Samples were vigorously mixed by vortexing for 1 minute at maximum speed, tenfold serial dilutions were prepared from each sample using the same saline solution, and finally, the dilutions were used for the detection and enumeration of different bacterial groups. To begin, 1 ml from each of the previously prepared dilutions was transferred into two separate sterile Petri-dishes to which approximately 15 ml of sterile melted and cooled plate count agar were added. After mixing, the inoculated plates, they were incubated in an incubator (Thermo Scientific 3951 Large Capacity, Germany) at 30°C for 48 hours. Total bacterial counts per gram were calculated on plates with 30-300 colonies and each count was recorded. In the next step, 1 ml from each of the previously prepared dilutions was transferred into two separate sterile plates to which approximately 15 ml of sterile melted and cooled Eosin methylene blue agar (EMB) medium was added. After mixing, the inoculated plates were incubated at 37°C for 48 hours. *Escherichia coli (E. coli)* spices, *Proteus* spices, and *Enterobacter* spices counts per gram were calculated based on the differential counts of green, colorless and pink colonies, respectively (Downes and Ito, 2001). Xylose Lysine Deoxycholate Agar (XLD) medium was used for the detection of *Salmonella* and red colonies with the black center were recorded for *Salmonella* species.

Statistical analysis

One-way analysis of variance was used to determine the effect of adding BSFP, BSFpr, and BSFL at levels of 2%, 4%, and 6% to the broilers' diet on chickens' growth performance, blood parameters, and humoral immunity. Data were statistically analyzed by the General Linear Model Procedure of the SAS software (SAS, 2004). Mean values were compared using Duncan's Multiple Range Test (Duncan, 1955) and significant differences were defined at $p < 0.05$.

RESULTS AND DISCUSSION

Productive performance

Table 2 shows the influence of inclusions levels and the form of BSF larvae on broiler performance. Both black soldier fly puré and BSFP supplementation improved broiler performance throughout the experimental period. Groups with 4% BSFP and 6% BSFPr had the highest body weight, while 6% BSFL had the lowest values ($p < 0.05$). At 3 and 5 weeks of age, chicks in the 4% BSFP group had the highest body weight, while different levels of BSFL recorded significantly lower values ($p < 0.05$). A similar pattern was recorded in body weight gain throughout all periods of the study. Concerning growth rate, 4% BSFP also had the highest percentage throughout the first 3 weeks and the entire experiment, but 4% BSFL recorded the highest percentage between 3-5 weeks of age. The previous research attributed the improvement in chicks' performance to the amino acid profile and higher protein content, compared to vegetable protein sources, which makes BSFP a desirable feed. However, the nutrient composition of BSFP fluctuates (Wang and Shelomi, 2017) since larvae are raised on a variety of sub-states, such as organic waste streams, by-products, chicken faces, and kitchen wastes leading to a wide range of nutritive value, crud protein (33-55%), ether extract (18-32%), calcium (2.4-5.8%), lysine (1.9-2.7%), and methionine (1.9-2.7%, Finke, 2013; Nguyen et al., 2015; Shumo et al., 2019). Results in the present study agree with those reported by Dabbou et al. (2018) who found an improvement in broiler performance with BSF inclusion. The positive modulation of live body weight (LBW) by dietary BSF inclusion partially agrees with what was reported by Oluokun (2000) and Loponte et al. (2017) who observed improved growth rate and higher LBW in chicks and Barbary partridges fed with *hermetia illucens* meal as a component of a complete diet and as a partial replacement (25% or 50%) of soybean meal. This is also in agreement with the successful use of BSF and prepupae grown on swine manure or kitchen waste as feed additives in young chicks. Attivi et al. (2020) found lower body weight gain (BWG) at 2%, 4%, and 6% BSF inclusion while the higher BWG was observed at 8% BSF inclusion.

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Table 2. The influence of different forms and levels of black soldier fly on body weight, body weight gain, and growth ratio of broiler chickens

Treatment	Body weight (g)			Body weight gain (g)			Growth rate (%)		
	1 Weeks	3 Weeks	5 Weeks	1-3 Weeks	3-5 Weeks	1-5 Weeks	1-3 Weeks	3-5 Weeks	1-5 Weeks
BSFP 2%	174.62 ± 1.64	761.86 ± 5.20 ^{f}	1940.98 ± 14.43 ^{d}	586.94 ± 7.05 ^{f}	1168.52 ± 1.97 ^{c}	1765.60 ± 0.73 ^{d}	125.38 ± 1.14 ^{f}	87.24 ± 1.4 ^{d}	166.97 ± 0.13 ^{c}
BSFP 4%	177.96 ± 1.64	831.63 ± 5.76 ^{a}	2000.13 ± 14.61 ^{a}	653.55 ± 7.05 ^{a}	1178.66 ± 1.97 ^{a}	1822.07 ± 10.73 ^{a}	129.49 ± 1.14 ^{a}	82.53 ± 1.4 ^{i}	167.32 ± 0.13 ^{a}
BSFP 6%	173.36 ± 1.64	809.34 ± 6.17 ^{b}	1913.82 ± 14.99 ^{f}	630.30 ± 7.05 ^{b}	1107.56 ± 1.97 ^{h}	1737.85 ± 0.73 ^{f}	128.63 ± 1.14 ^{b}	81.85 ± 1.4 ^{j}	166.73 ± 0.13 ^{e}
BSFPr2%	174.40 ± 2.02	786.59 ± 15.88 ^{d}	1955.66 ± 17.11 ^{b}	611.98 ± 13.66 ^{d}	1168.90 ± 17.05 ^{b}	1780.88 ± 5.75 ^{b}	127.40 ± 1.06 ^{h}	85.27 ± 1.59 ^{g}	167.24 ± 0.21 ^{b}
BSFPr4%	177.96 ± 2.02	793.85 ± 16.28 ^{c}	1945.13 ± 16.89 ^{c}	615.89 ± 13.66 ^{c}	1151.28 ± 17.05 ^{e}	1767.17 ± 5.75 ^{c}	126.75 ± 1.06 ^{d}	84.07 ± 1.59 ^{h}	166.47 ± 0.21 ^{f}
BSFPr6%	173.36 ± 2.02	772.79 ± 15.51 ^{e}	1923.95 ± 16.08 ^{e}	595.84 ± 13.66 ^{e}	1155.18 ± 17.05 ^{d}	1751.02 ± 5.75 ^{e}	126.14 ± 1.06 ^{e}	85.90 ± 1.59 ^{f}	166.94 ± 0.21 ^{d}
BSFL 2%	174.40 ± 7.21	618.08 ± 12.75 ^{h}	1759.36 ± 12.97 ^{h}	443.32 ± 16.79 ^{h}	1141.23 ± 19.06 ^{f}	1584.55 ± 7.37 ^{h}	111.71 ± 2.52 ^{b}	96.09 ± 2.23 ^{b}	163.91 ± 0.86 ^{h}
BSFL 4%	191.73 ± 7.21	589.46 ± 13.09 ^{i}	1691.67 ± 13.50 ^{i}	398.67 ± 16.79 ^{i}	1101.26 ± 19.06 ^{i}	1499.93 ± 7.37 ^{i}	101.84 ± 2.52 ^{c}	96.58 ± 2.23 ^{a}	159.31 ± 0.86 ^{j}
BSFL6%	173.36 ± 7.21	570.00 ± 13.27 ^{j}	1608.29 ± 13.69 ^{j}	396.65 ± 16.79 ^{j}	1038.54 ± 19.06 ^{j}	1435.18 ± 7.37 ^{j}	106.43 ± 2.52 ^{i}	95.50 ± 2.23 ^{c}	161.09 ± 0.86 ^{i}
Control	175.64 ± 2.04	744.86 ± 13.20 ^{g}	1871.81 ± 19.33 ^{g}	568.00 ± 12.56 ^{g}	1126.94 ± 23.60 ^{g}	1694.94 ± 31.52 ^{g}	123.24 ± 3.41 ^{g}	86.13 ± 0.98 ^{e}	165.46 ± 4.06 ^{g}
P-value	0.3283	0.0001	0.0001	0.0001	0.2229	0.0001	0.0003	0.0001	0.7456

Values with different superscripts within a column are significantly different ($p \leq 0.05$). BSFP: Black soldier fly powder, BSFPr: Black soldier fly puré, BSFL: Black soldier fly larvae

Table 3. The influence of different forms and levels of black soldier fly on plasma total protein, total cholesterol, triglycerides, low-density lipoproteins, high-density lipoproteins, uric acid, Triiodothyronine, and Thyroxine hormones of boiler chickens

Treatment	T.P (g/dl)	Al (g/dl)	TCh (mg/dl)	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	UA (mg/dl)	T3 (mg/dl)	T4 (mg/dl)
BSFP 2%	7.83^{a}	3.45^{g}	303.36^{a}	88.89^{g}	207.08^{c}	78.51^{a}	3.27^{j}	6.07^{f}	2.56^{c}
BSFP 4%	5.94^{b}	3.16^{i}	286.24^{e}	96.76^{e}	218.27^{a}	48.62^{i}	5.00^{a}	6.53^{c}	2.58^{a}
BSFP 6%	3.37^{j}	3.52^{f}	266.67^{g}	111.11^{b}	182.72^{h}	61.72^{f}	4.00^{e}	7.17^{a}	2.55^{d}
Mean Standard Error	± 0.57	± 0.15	± 7.14	± 8.79	± 6.8	± 3.94	± 0.44	± 0.21	± 0.02
BSFPr 2%	4.42^{f}	3.40^{h}	288.69^{c}	68.52^{i}	202.45^{e}	72.53^{c}	3.43^{h}	5.57^{i}	2.57^{b}
BSFPr 4%	3.87^{i}	3.12^{j}	283.18^{f}	93.98^{f}	207.03^{d}	57.36^{g}	3.52^{g}	5.80^{h}	2.55^{d}
BSFPr 6%	4.23^{g}	3.73^{d}	258.72^{i}	77.78^{h}	201.67^{f}	41.49^{j}	4.73^{b}	6.30^{d}	2.51^{f}
Mean Standard Error	± 0.32	± 0.16	± 14.72	± 6.79	± 13.26	± 4.91	± 0.47	± 0.23	± 0.02
BSFL 2%	4.65^{d}	3.87^{c}	263.61^{h}	106.94^{d}	178.89^{i}	63.33^{e}	4.11^{c}	6.97^{b}	2.42^{i}
BSFL 4%	4.48^{e}	3.90^{b}	245.26^{j}	107.87^{c}	147.82^{j}	75.86^{b}	3.80^{f}	5.33^{j}	2.49^{g}
BSFL 6%	4.98^{c}	3.95^{a}	287.46^{d}	107.87^{c}	199.11^{g}	66.78^{d}	3.36^{i}	6.03^{g}	2.45^{h}
Mean Standard Error	± 0.67	± 0.16	± 8.04	± 9.89	± 10.3	± 3.25	± 0.43	± 0.41	± 0.02
Control	4.12 ± 0.36 ^{h}	3.71 ± 0.09 ^{e}	291.74 ± 13.93 ^{b}	145.83 ± 6.9 ^{a}	207.86 ±6.30 ^{b}	54.71 ± 3.30 ^{h}	4.04 ± 0.22 ^{d}	6.10 ± 0.33 ^{e}	2.52 ± 0.04 ^{e}
P-value	0.0122	0.0004	0.5781	0.0001	0.0033	0.0245	0.7189	0.4912	0.3295

Values with different superscripts within a column are significantly different ($p \leq 0.05$). BSFP: Black soldier fly powder, BSFPr: Black soldier fly puré, BSFL: Black soldier fly larvae; TP: Total protein, AL: Albumin, TCh: Total cholesterol, TG: Triglycerides, UA: Uric acid, T3: Triiodothyronine, T4: Thyroxine, LDL: Low-density lipoproteins, HDL: High-density lipoproteins

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Blood plasma constituents

The influence of different forms of black soldier fly supplementation on blood plasma constituents is shown in Table 3. The substitution of 2% BSFP increased total protein ($p < 0.05$), HDL ($p < 0.05$), and total cholesterol ($p > 0.05$), but decreased uric acid level ($p > 0.05$). Moreover, 4% BSFP substitution increased LDL significantly ($p < 0.05$) and thyroid hormone T4 insignificantly ($p > 0.05$), and decreased HDL level significantly ($p < 0.05$). However, the lowest level of total protein and the highest level of triiodothyronine were observed in 6% BSFP, on the other hand, 4% and 6% BSFPr caused the lowest level of triglycerides ($p < 0.05$) and HDL level, respectively ($p < 0.05$). The highest albumin and triglycerides levels were recorded at 6% BSFL and control groups, respectively ($p < 0.05$). According to the result of Attivi et al. (2020), any BSF meal inclusion level below 8% had a negative impact on protein digestion. The delivery of BSF larval meal to Japanese broiler quails had a substantial impact on physiological indexes ($p < 0.05$, Dörper et al., 2021). The present results are partially agreed with those reported by Marono et al. (2017) and Loponte et al. (2017) that BSF incorporation in the diet has been shown to improve the blood profile of laying hens. On the contrary, Schiavone et al. (2017b) reported the replacement of soybean oil in their diet with 50% or 100% BSF larvae had no effect on the blood profile of Ross 308 broilers at 35 days of age.

Humoral immune response

The effect of dietary various BSF larvae levels on the humoral immunity (HI) antibody titers against Newcastle disease virus is presented in Table 4. The HI antibody titer fluctuated among groups from age to age. At 18 days of age, 2% BSFPr, 4% BSFPr, and 6% BSFL levels had the highest titer. At 23 days of age, 2% BSFP, 4% BSFPr, and 6% BSFPr had the highest titer while 4% BSFP had the lowest. At 28 days of age, the highest values were recorded for 2% BSFL, 4% BSFP, and 4% BSFPr levels. Previous research demonstrates that some amino acids and peptides in BSFL feeding can help the experimental quails' immune systems (Harlystiarini et al., 2020). According to Rumpold and Schlüter (2013), BSFL as an insect should be used as a source of animal protein in chicken production. Insects could be useful in the production of a variety of bioactive chemicals with beneficial qualities. Insects are beneficial not only as a rich source of protein in food and feed supply but also as a source of pharmacological and medicinal compounds that could preserve life and livestock from a variety of ailments (Hirose et al., 2013). For the _ENREF_43 present study no immunostimulant drug was used, therefore, it was assumed that the chicks naturally expressed their antibodies against the Newcastle virus.

Table 4. The influence of different forms and levels of black soldier fly on humoral immunity antibody titer against Newcastle disease virus in broiler chickens

Newcastle disease virus titer / Treatment	18 Days	23 Days	28 Days
BSFP 2%	4.00 ± 0.85 ^{h}	7.00 ± 0.75 ^{a}	6.00 ± 0.63 ^{f}
BSFP 4%	5.00 ± 0.85 ^{g}	4.33 ± 0.75 ^{f}	8.67 ± 0.63 ^{b}
BSFP 6%	5.33 ± 0.85 ^{f}	6.33 ± 0.75 ^{b}	6.67 ± 0.63 ^{e}
BSFPr 2%	9.00 ± 0.81 ^{a}	5.00 ± 0.42 ^{e}	6.00 ± 0.39 ^{f}
BSFPr 4%	8.00 ± 0.81 ^{c}	6.33 ± 0.42 ^{b}	8.00 ± 0.39 ^{c}
BSFPr 6%	7.33 ± 0.81 ^{d}	7.00 ± 0.42 ^{a}	4.67 ± 0.39 ^{h}
BSFL 2%	4.00 ± 0.57 ^{h}	5.00 ± 0.80 ^{e}	9.00 ± 0.37 ^{a}
BSFL 4%	4.00 ± 0.57 ^{h}	5.67 ± 0.80 ^{d}	7.33 ± 0.37 ^{d}
BSFL 6%	8.33 ± 0.57 ^{b}	6.00 ± 0.80 ^{c}	5.33 ± 0.37 ^{g}
Control	5.67 ± 0.42 ^{e}	6.00 ± 0.30 ^{c}	6.67 ± 0.50 ^{e}
P-value	0.0001	0.5889	0.4904

Values with different superscripts within a column are significantly different ($p \leq 0.05$). BSFP: Black soldier fly powder, BSFPr: Black soldier fly puré, BSFL: Black soldier fly larvae, HI: Humoral immunity, NDV: Newcastle disease virus, DS: Days of age.

Intestinal bacteriological counts

Beneficial bacteria and *Proteus* species results are shown in Table 5. Generally, dietary inclusions with BSF larvae significantly promoted the proliferation of benefits, such as bacteria microbiota, except for both 2% BSFP and 2% BSFPr ($p < 0.05$). On the other hand, the inclusion of BSFP 2% inhibits *Proteus* species more than the other groups. Moreover, the inclusion of 6% BSFP, 2% BSFPr, 4% BSFPr, 6% BSFPr, 2% BSFL, 4% BSFL, 6% BSFL and control did not significantly inhibit *E. coli* proliferation ($p > 0.05$), at the same time, they significantly inhibited *Enterobacter* species proliferation ($p < 0.05$). However, BSFP at levels of 2% and 4% promoted the proliferation of *E. coli* and *Enterobacter* species, respectively. The antibacterial effectiveness of BSF larvae extracts against *Salmonella* species and *E. coli* species as Gram-negative bacteria was demonstrated by Harlystiarini et al. (2020) who discovered that the methanol extract of BSF larvae was a little more susceptible to *E. coli* bacteria versus Gram-positive bacteria. This

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difference in sensitivity could be due to a change in how ribosomes or other components from bacteria's membrane cells interact with the active chemical in BSFL (Choi et al., 2012). The effect of a pharmaceutical extract of BSFL was studied on *E. coli* by Choi et al. (2012). After the larvae were extracted using various types of organic solvents, antibacterial activity was determined using the agar disc diffusion method and turbidimetry assay. The antibacterial activity of the methanol extract (ME) was demonstrated against the proliferation of Gram-negative bacteria, such as *Shigella sonnei*, *Klebsiella pneumoniae*, and *Neisseria gonorrhoeae*. Gram-positive bacteria, including *Bacillus subtilis*, *Streptococcus mutans*, and *Sarcina lutea*, on the other hand, had no antibacterial effect. According to (Pan et al., 2009), the inhibition zone diameter created by BSF larvae extract was over 6 mm, indicating high antibacterial action for both species. Furthermore, BSF larvae extract had slightly better antibacterial activity against *Salmonella* species than it did against *E. coli*. Antimicrobial peptides (AMPs) were synthesized in the fat body and subsequently released into the hemolymph to provide humoral immunity (Tsakas and Marmaras, 2010). The AMPs molecule was detected in all living creatures, including bacteria and humans. Antimicrobial peptides were an element of the innate immune system of insects, among which defensin has been identified in insects (Zasloff, 2002). It was indicated that the creation of a channel in the cytoplasm membrane of bacteria was the general mode of action of insect defensin (Yi et al., 2014). Cardiolipin, the major phospholipid in bacteria, has a strong affinity for defensin. This interaction between defensin and phospholipid may induce microheterogeneity in the lipid membrane, which may be associated with the creation of channels that are responsible for the defensin's biological activity (Yi et al., 2014). Chitin and lauric acid in black solidier fly work as antimicrobial compounds against harmful bacteria thtough their cationic characteristic that hold harm bacteria prevent them to penetrate the call membrane (Je and Kim, 2006; Yi et al., 2014) .

Table 5. The influence of different forms and levels of black soldier fly on intestinal bacterial counts in broiler chickens

Treatment	Beneficial bacteria	*Escherichia coli*	*Proteus* species	*Enterobacter* species
BSFP 2%	9.27 ± 0.15^{c}	6.33 ± 0.16	-	5.49 ± 0.11
BSFP 4%	9.64 ± 0.15^{abc}	6.56 ± 0.16	4.88 ± 0.26	6.18 ± 0.11
BSFP 6%	9.74 ± 0.15^{abc}	-	5.12 ± 0.26	-
BSFPr 2%	8.31 ± 0.15^{d}	-	4.25 ± 0.26	-
BSFPr 4%	9.41 ± 0.15^{bc}	-	5.05 ± 0.26	-
BSFPr 6%	9.91 ± 0.15^{a}	-	4.48 ± 0.26	-
BSFL 2%	9.46 ± 0.15^{abc}	-	4.38 ± 0.26	-
BSFL 4%	9.68 ± 0.15^{abc}	-	4.35 ± 0.26	-
BSFL 6%	9.84 ± 0.15^{ab}	-	4.88 ± 0.26	-
Control	9.35 ± 0.15^{c}	-	5.03 ± 0.26	-
P-value	0.0001	0.3231	0.1245	0.0014

Values with different superscripts within the column are significantly different ($p \leq 0.05$). BSFP: Black soldier fly powder, BSFPr: Black soldier fly puré, BSFL: Black soldier fly larvae.

CONCLUSION

It was concluded that BSF larvae with its two forms (black soldier fly powder and black soldier fly puré) can be used as a protein source rather than soybean meal and the deleterious effects were not seen on broiler chickens during the present study. The two forms of larvae improved immunological status and suppressed *E. coli*, *Enterobacter*, and *protues* count. The BSF larvae could increase the beneficial bacteria in the intestine of broiler chickens notably. The black soldier fly powder at a level of 4% was recommended which can be replaced with soybean content in broiler chicken diets.

DECLARATIONS

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Authors' contribution

Ahmed El-Kaiaty and Abd El-Rahman Atta designed the study and the experiment was carried out by Doha Dawa. The article was written under the supervision of Ahmed El-Kaiaty and Abd El-Rahman Atta. Tarek Ragab El-Sayed made the microbiological examination. The data were analyzed by Hamada Okasha, lecturer of poultry management under the guidance of Abd El-Rahman Atta. All authors checked and confirm the final analyzed data and the revised manuscript.

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Competing interests

The authors state that they all have no potential conflicts in relation to this research, writing, or publishing.

Ethical considerations

The research had all credibility and trust and did not plagiarise or copy from any other papers or ideas. The present findings did not have any fabrication or falsification. The authors consent to publish only in World's Veterinary journal and did not submit this article or any part of the present scientific results in any other journals. All the data carried out from the trial and writing were supervised by the supervisors (Ahmed El-Kaiaty and Abd El-Rahman Atta).

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