Research Article

Impact of Dietary Inclusion of Black Soldier Fly Larvae (*Hermetia illucens*) as a Replacement for Soybean-Corn Ingredients on Egg Production, Physiological Status, and Economic Efficiency of Laying Hens

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Abstract | In the recent years, insect meals have been introduced as efficient and inexpensive sources of protein and energy in poultry nutrition. The current study was conducted to explore the possible impact of soybean-corn replacement with various levels of black soldier fly larvae *Hermetia illucens* (BSFL) meal on egg production, egg quality, physiological aspects and economic efficiency of laying hens. The study employed 270 commercial layers, 40-wk-old, that belonged to the W-36 *Hy-Line* chickens. The layers were randomly designated into five equal treatment groups (9 replicates × 6 hens per replicate for each treatment) according to the dietary inclusion levels of BSFL meal. The first group of birds were fed a basal diet of soybean-corn meals and served as a control (0% BSFL meal), while the remaining 4 groups were fed a basal diet in which the soybean-corn meals were partially replaced with 3%, 6%, 9%, and 12% BSFL meals, respectively. The experimental trial continued for 10 consecutive weeks from 40 to 50 wk of age. One-way analysis of variance (ANOVA) including a polynomial test was carried out to explore the linear and quadratic contrasts of increasing the BSFL levels on all the parameters. The results of this study showed a linear improvement ($p < 0.05$) in the egg production (by 3.38 percent points than control), egg weight (by 1.54 g than control), feed conversion (by 20% than control), and egg quality traits, such as Haugh unit, yolk color, shell strength, and shell thickness, with the increase in the BSFL inclusion levels into the layer diets. The BSFL treatment linearly ($p < 0.05$) augmented the concentration of total protein, triglycerides, cholesterol, and calcium in the plasma. Furthermore, a linear increasing effect ($p < 0.05$) on the T_3 and E_2 hormone concentrations and on the humoral and cellular immune response were obtained as the dietary BSFL level increased. Moreover, BSFL treatment linearly increased the profit margin, the cost benefit ratio and the return on investment per bird, recording the highest economic efficiency when employing 12% BSFL in the layer diets. Our results conclude that each 3% of the soybean meal in the laying hen's diet can be replaced by 1% of the BSFL meal to achieve favorable outcomes on the performance, the physiological mechanisms, and the economic profits of egg production.

Keywords | *Hermetia illucens*, Larvae meal, Laying hens, Egg production, Egg quality, Physiological aspects, Economic efficiency

Received | December 31, 2022; **Accepted** | January 09, 2023; **Published** | January 31, 2023
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Citation | Nassar FS, Alsahlawi AM, Abbas AO, Alaqil AA, Kamel NN, Abdelwahab AM (2023). Impact of dietary inclusion of black soldier fly larvae (*Hermetia illucens*) as a replacement for soybean-corn ingredients on egg production, physiological status, and economic efficiency of laying hens. Adv. Anim. Vet. Sci. 11(2):295-304.
DOI | https://dx.doi.org/10.17582/journal.aavs/2023/11.2.295.304
ISSN (Online) | 2307-8316

INTRODUCTION

Poultry products have been considered as essential substances in human nourishment for their high nutritional value. However, augmented populations, climate changes, spread of diseases, and other possible constraints may drive small-scale producers out of the business, and consequently, lead to increasing trends in poultry feed costs. It is well-known that corn and soybean meals represent more than 60% of poultry ration ingredients to supply the amounts of protein and energy required for intensive production (Ahiwe et al., 2018). Therefore, adopting available, efficient and inexpensive alternative sources of protein and energy in poultry feeds may be a sustainable option in poultry industry (van Huis, 2013).

In the last decade, utilizing insects, such as yellow mealworm (*Tenebrio molitor*), common housefly (*Musca domestica*), house cricket (*Acheta domesticus*), black soldier fly (*Hermetia illucens*), and others, in poultry feed has gained a wide interest due to their high contents of protein, loaded with essential amino acids compared to conventional feedstuffs (Al-Qazzaz and Ismail, 2016). The black soldier fly larvae (BSFL) have been portrayed by their enriched contents of protein (35-60%), energy (7-42% fats), essential amino acids (0.08-0.90% methionine, 1.3% methionine+ cysteine, 0.34-3.30% lysine, 0.22-2.26% threonine, and 0.33-3.38% valine), fatty acids (especially lauric acid and palmitic acid), and minerals (0.21-4.39% Ca and 0.74-0.95% P) (Nyakeri et al., 2017; Kawasaki et al., 2019), besides their safety from any disease-causing agents compared with other insect meals (Eilenberg et al., 2015). Additional advantages have been reported from the use of BSFL, such as diminishing of animal manure mass, methane formation, off-gassing, houseflies' populations, and odors (Veldkamp and Bosch, 2015).

The BSFL meal have been successfully employed in broiler diets to improve their performance, feed efficacy, carcass traits, meat features, and intestinal microbiota (de Marco et al., 2015; Schiavone et al., 2017, 2018; Mohammed, 2018; Neumann et al., 2018; Pieterse et al., 2019; Biasato et al., 2020; de Souza Vilela et al., 2021). In laying hens, the results obtained after BSFL insertion in the diets were widely variable, depending on the line, age, doses, duration, and manufacturer processing (Elahi et al., 2022). Maurer et al. (2016) reported that BSFL meal included as a full source of protein into layer diets did not adversely affect the layer production or feed efficiency. Furthermore, it was found that dietary supplementation of BSF or BSFL meals at different levels (ranged 3-15%) to laying hens improved egg production (Liu et al., 2021), egg weight and egg mass (Kawasaki et al., 2019; Heuel et al., 2021), albumin height, Haugh unit, yolk color, and shell thickness (Mwaniki et al., 2018; Kawasaki et al., 2019; Liu et al., 2021), plasma calcium (Kawasaki et al., 2019), immunoglobin production, glutathione peroxidase, and superoxide dismutase activity, and inhibited lipid peroxidation (Chu et al., 2020; Liu et al., 2021). Moreover, Marono et al. (2017) demonstrated that laying hens fed on soybean-meal diet replaced with 17-100% BSF meal increased the percentage of small and large size eggs, blood globulin and blood calcium, while it compromised the growth and egg production, and decreased blood lipids, chloride, and creatine. In contrast, Attivi et al. (2022) concluded that partial or full replacement of fish meal in a layer diet with 75-100% BSFL meal substantially improved the body weights and feed efficiency, and increased the total protein, total cholesterol, triglycerides, and T_3 hormone concentrations.

Although considerable studies have been reported on the feeding value of the BSFL meal in meat- and egg-type chickens, there is still a need to explore the effectiveness, the functional mechanisms, the side effects, and the feasibility of its application. Particularly, there is a shortage of research discussing the use of BSFL meal as an alternative of the traditional protein sources in the nourishment of laying hens. Thus, the goal of the present study was to highlight the potential impact of inserting various levels of BSFL meal as a replacement for the soybean-corn meal in the diet on egg production, quality traits, physiological profile, and economic efficiency of laying hens.

MATERIALS AND METHODS

ETHICAL APPROVAL

The experimental study protocol was approved by the Research Ethics Committee at King Faisal University Ethics Committee (KFU-REC-2022-AUG-ETHICS362).

BIRDS AND TREATMENTS

The study employed 270 commercial layers at 40-week-old belonging to the W-36 *Hy-Line* chickens. The layers were raised in individual cages in an open housing system equipped with standard environmental and hygienic facilities. The hens were exposed to 16 h light followed by 8 h dark daily, while feed and water were provided *ad libitum*. The layers were assigned at random into five treatment groups (9 replicates of 6 hens each) according to the dietary inclusion levels of BSFL meal. The first group of birds were fed a basal diet of soybean-corn meals and served as a control (0% BSFL meal), while the remaining 4 groups of birds were fed a basal diet in which the soybean-corn meal was partially replaced with 3%, 6%, 9%, and 12% BSFL meals, respectively. The experimental trial continued for 10 consecutive weeks from 40 to 50 wk of age. The experimental diets were composed to be isonitrogenous, isocaloric, and as a mash, following the nutritional recommendation of the W-36 *Hy-Line* layers (Table 1). The nutritional values of the formulated diets were determined using the AOAC guidelines (AOAC, 2005).

Table 1: Ingredients and nutritional values of the experimental diets including a black soldier fly larvae (BSFL) meal.

Ingredients (g/kg as fed)	**BSFL meal groups**				
	0%	**3%**	**6%**	**9%**	**12%**
BSFL meal	0.0	30.0	60.0	90.0	120.0
Soybean meal	274.0	244.0	215.0	195.0	175.0
Yellow corn	567.5	567.5	566.5	559.5	549.5
Wheat bran	10.0	10.0	10.0	10.0	10.0
Soybean oil	30.0	30.0	30.0	30.0	30.0
Bone meal	30.0	30.0	30.0	30.0	30.0
Limestone	80.0	80.0	80.0	80.0	80.0
Salt (NaCl)	4.0	4.0	4.0	4.0	4.0
Premix [1]	3.0	3.0	3.0	3.0	3.0
Methionine	1.5	1.5	1.5	1.5	1.5
Calculated nutrients					
Metabolizable energy (MJ/kg)	12.6	12.6	12.6	12.6	12.6
Crude protein (g/kg)	179.7	179.7	179.7	179.7	179.7
Calcium (g/kg)	40.2	40.2	40.2	40.2	40.2
none phytase phosphorus (g/kg)	5.2	5.2	5.2	5.2	5.2
Lysine (g/kg)	9.5	9.5	9.5	9.5	9.5
Methionine (g/kg)	4.2	4.2	4.2	4.2	4.2
Determined nutrients					
Crude protein (g/kg)	176.1	176.7	174.2	174.9	175.5
Crude fat (g/kg)	66.0	64.5	63.8	62.1	61.5
Crude fiber (g/kg)	47.0	46.5	46.5	45.8	45.5
Calcium (g/kg)	39.5	39.8	40.1	40.5	41.0
none phytase phosphorus (g/kg)	4.7	4.4	4.3	4.1	4.0

[1] Premix provide the following components per kg of the experimental diet: vitamin A= 8000 IU; vitamin D_3= 1500 IU; vitamin E= 15 mg; vitamin K= 2 mg; riboflavin = 4 mg; cobalamin= 10 µg; choline= 500 mg; niacin= 25 mg; manganese= 60 mg; zinc= 50 mg.

The BSFL meal was obtained as a whole-dried larvae from a commercial manufacturer (Enterra Grubs™, Maple Ridge, BC, Canada) and were finely ground with a blender. The BSFL powder was first mixed with a part of the diet manually, then added to the rest of the diet to reach the final BSFL concentration in the experimental diets. The chemical composition of the BSFL, soybean (SB) and corn (C) meals were presented in Table 2.

Table 2: The nutritional composition (per 100 g) of black soldier fly larvae (BSFL), soybean (SB) and corn (C) meals*.

Item	**BSFL meal**	**SB meal**	**C meal**
Proximate			
Moisture	5 g	10 g	14 g
Crude protein	34 g	44 g	7.5 g
Fat (ether extract)	40 g	0.5 g	3.5 g
Crude fiber	10 g	7.0 g	1.9 g
Gross energy	2.3 MJ	1.7 MJ	1.9 MJ
Calcium	800 mg	250 mg	10 mg
Phosphorus	600 mg	200 mg	120 mg
Essential amino acids			
Isoleucine	1.6 g	2.5 g	0.29 g
Leucine	2.5 g	3.5 g	-
Valine	2.6 g	2.4 g	0.42 g
Lysine	2.2 g	2.8 g	0.24 g
Tryptophan	0.5 g	0.6 g	0.07 g
Phenylalanine	1.4 g	2.3 g	-
Methionine	0.5 g	0.7 g	0.18 g
Threonine	1.3 g	1.7 g	0.29 g
Non-essential amino acids			
Cysteine	0.3 g	0.7 g	0.18 g
Tyrosine	2.2 g	1.7 g	-
Alanine	2.2 g	2.0 g	-
Arginine	1.7 g	3.4 g	0.40 g
Aspartic acid	3.0 g	-	-
Glutamic acid	3.9 g	-	-
Glycine	1.8 g	-	-
Histidine	1.0 g	-	-
Proline	2.3 g	-	-
Serine	1.4 g	-	-

* According to the commercial supplier (not analyzed).

PRODUCTIVE PERFORMANCE

The layers in each treatment group were weighed at the 40th and the 50th wk of age to determine the initial (IBW) and the final body weight (FBW), respectively. Data of egg number and weight were recorded every day to evaluate the average egg production (EP%) and egg weight (EW) per hen during the experimental period. The average daily feed intake (FI) was assessed per hen in each treatment. Feed conversion ratio (FCR) was then determined per hen by calculating the FI per unit egg mass.

EGG QUALITY

Thirty eggs from each treatment group were randomly collected at the end of the experiment for evaluation of the quality traits according to a previous study (Abbas et al., 2022). Egg weight was detected (W) before breaking to separate the albumen and the yolk. The albumen height

(AH) was measured using a tripod micrometer (Baxlo Instrumentos de Medida y Precisión, SL, Barcelona, Spain), and the Haugh unit was calculated [HU = 100 log (AH − 1.7 $W^{0.37}$ + 7.6)]. The egg yolk color (YC) was scored physically using DSM-YC Fan (ORKA Food Technology, LLC, Utah, USA). The eggshell was washed and let dry in air. A specific gauge was used to measure the shell thickness (ST) (Baxlo), while the shell strength (SS) was measured by an egg force reader system (ORKA).

Physiological parameters

After termination of the trials (50 wk of age), blood samples were taken from the brachial vein of 2 hens per replication in each treatment group at 3:00-5:00 pm and placed into a heparinized tube. Plasma was separated by a centrifugation (2000× g; 10 min; 4°C) and kept at −20 °C for further assays. The total proteins (TP), triglycerides (TG), total cholesterols (CH), and calcium (CA) levels in the plasma were measured according to the protocol description of commercial colorimetric kits (Abcam, Waltham, MA, USA; Catalog no. ab102535, ab65336, ab282928, and ab102505, respectively) using a spectrophotometer (CE1010, Cecil Instruments Limited, Cambridge, UK). While the levels of triiodothyronine (T3) and estradiol (E2) hormones were determined in the plasma using chicken's ELISA kits obtained from MyBioSource (Catalog no. MBS269454 and MBS701593, respectively; San Diego, CA, USA) and processed using a microplate reader (ELx808™, BioTek Instruments, Winooski, VT, USA).

Two hens per replication in each treatment group were tested for the humoral immune response. Antibody titers were assessed using sheep red blood cells (SRBC) according to the methods outlined in a prior study (Bhatti et al., 2017) with a slight modification. One week before the end of the treatments, the birds were injected with 1 mL of washed-diluted SRBC (5% v/v in saline solution). Blood sera were then collected from birds using centrifugation. Ten serial doubling dilutions of sera were prepared in a 96-well tray using phosphate buffer saline (PBS) solution. Each well was pipetted with 2% SRBC then incubated at 37°C for 30 min. The Ab titers were reported as $\log_2$ of the reversed values of the last dilution showing positive agglutination.

Furthermore, the cellular immunity was evaluated using procedures of a previous work (Al-Khalifa, 2016). In brief, a specific dermal region of the wattle of 2 hens per replication in each treatment group were injected with 0.5 mg phytohemagglutinin (PHA, Thermo Fisher Scientific, Waltham, MA, USA) dissolved in 0.1 mL PBS. The increase in the wattle swelling (as a positive response to the PHA-test) was estimated 1-day post injection using a micrometer.

Economic efficiency

The economic efficiency of the partial replacement of SB and C meals with BSFL meal into the layer diet was evaluated based on the cost benefit ratio (CBR) and the return on investment (RoI) analyses (Onsongo et al., 2018). The feed cost was the only factor considered in the analysis while the other cost factors, including labor, medication, water, electricity, housing, etc., were presumed to be similar for all the treatments. Feed costs were estimated based on the quantity of ingredients in each experimental diet and their prices during the time of experiment. The benefits were considered as the revenue collected from sale of the eggs produced during the experimental period. The CBR represents the ratio between the total revenue and the total cost of the production. The RoI measures the gain/loss of the invested money. The economic efficiency (EE) was calculated according to the gain/loss of money investment before and after BSFL meal inclusion into the layer diets.

Statistical analysis

Data were analyzed using the SPSS software package (version 22.0; IBM corp., NY, USA, 2013). One-way analysis of variance (ANOVA) including a polynomial test was carried out to explore the linear and quadratic contrasts of increasing the BSFL levels into layer diets on all the parameters of productive performance, egg quality, physiological aspects, and economic efficiency. The bird was considered as the experimental unit for the analysis of productive performance (n = 54), physiological parameters (n = 18), and economic analysis (n = 54), while the egg was considered as experimental unit for egg quality traits (n = 30). Means and the pooled standard error of means (SEM) for groups were presented and separated using Tukey's post hoc test considering the level of statistical significance at $p < 0.05$.

RESULTS

Productive performance

Results of the productive performance as affected by BSFL meal inclusion into layer diets are shown in Table 3. The BSFL diets did not affect the FBW and FI of the layers. There was a linear increase ($p < 0.05$) in the EP and EW, and a linear and quadratic decrease in the FCR with the increase in the BSFL inclusion levels into the layer diets.

Egg quality

The egg quality traits as affected by BSFL meal inclusion into layer diets are shown in Table 4. The egg HU and YC were linearly and quadratically increased ($p < 0.05$) as a result to the increase in the dietary BSFL levels. In addition, the BSFL treatment linearly ($p < 0.05$) increased the egg ST and SS.

Table 3: Effect of dietary black soldier fly larvae (BSFL) meal on the productive performance of laying hens.

Dietary BSFL meals	IBW, g	FBW, g	FI, g/d	EP, %	EW, g	FCR
0%	1550.69	1600.79	99.67	89.23^{c}	61.27^{b}	1.82^{ab}
3%	1552.08	1590.35	101.11	89.07^{c}	61.22^{b}	1.85^{a}
6%	1549.86	1600.79	100.56	91.60^{b}	61.47^{b}	1.79^{b}
9%	1550.09	1596.48	101.00	92.86^{a}	62.81^{a}	1.73^{c}
12%	1551.67	1594.52	99.56	92.61^{a}	62.87^{a}	1.71^{c}
SEM	7.257	8.854	0.758	0.300	0.269	0.014
P-value						
Combined	0.997	0.736	0.136	< 0.001	< 0.001	< 0.001
Linear term	0.998	0.748	0.845	< 0.001	< 0.001	< 0.001
Quadratic term	0.884	0.925	0.022	0.078	0.075	0.020

Means within the same column (n = 54 birds per treatment group) presented with uncommon letters differ significantly at p-value < 0.05. IBW, initial body weight; FBW, final body weight; FI, feed intake; EP, egg production; EW, egg weight; FCR, feed conversion ratio. SEM = pooled standard error of means.

Table 4: Effect of dietary black soldier fly larvae (BSFL) meal on the egg quality of laying hens.

Dietary BSFL meals	HU, units	YC, score	ST, mm	SS, kg/cm²
0%	82.14^{b}	7.93^{d}	0.32^{c}	3.84^{b}
3%	85.15^{ab}	8.47^{d}	0.33^{bc}	3.95^{b}
6%	88.76^{a}	10.00^{c}	0.37^{b}	4.47^{a}
9%	87.22^{a}	11.47^{b}	0.44^{a}	4.66^{a}
12%	86.63^{ab}	12.87^{a}	0.45^{a}	4.73^{a}
SEM	1.658	0.247	0.016	0.143
P-value				
Combined	0.002	< 0.001	< 0.001	< 0.001
Linear term	0.004	< 0.001	< 0.001	< 0.001
Quadratic term	0.006	0.013	0.340	0.281

Means within the same column (n = 30 eggs per treatment group) presented with uncommon letters differ significantly at p-value < 0.05. HU, Haugh units YC, yolk color; ST, shell thickness; SS, shell strength. SEM = pooled standard error of means.

Table 5: Effect of dietary black soldier fly larvae (BSFL) meal on the plasma total protein, triglycerides, total cholesterols, and calcium concentrations of laying hens.

Dietary BSFL meals	TP, g/dL	TG, mg/dL	CH, mg/dL	CA, mg/dL
0%	3.99^{c}	173.55^{c}	120.60^{d}	24.21^{d}
3%	4.31^{c}	172.67^{c}	121.99^{d}	28.16^{c}
6%	5.30^{b}	189.07^{b}	135.88^{c}	31.29^{bc}
9%	6.71^{a}	188.13^{b}	143.79^{b}	33.05^{ab}
12%	6.54^{a}	199.78^{a}	151.30^{a}	35.97^{a}
SEM	0.306	1.557	2.187	1.153
P-value				
Combined	< 0.001	< 0.001	< 0.001	< 0.001
Linear term	< 0.001	< 0.001	< 0.001	< 0.001
Quadratic term	0.485	0.068	0.286	0.267

Means within the same column (n = 18 birds per treatment group) presented with uncommon letters differ significantly at p-value < 0.05. TP, total protein; TG, triglycerides; CH, cholesterols; CA, calcium. SEM = pooled standard error of means.

PHYSIOLOGICAL STATUS

The effects of dietary inclusion of the BSFL meals on the layer physiological parameters are summarized in Tables 5 and 6. The TP, TG, CH, and CA were linearly ($p < 0.05$) enhanced by increasing the levels of the BSFL meals into the layer diets (Table 5). There was a linear increasing response ($p < 0.05$) in the T_3 and E_2 hormone concentrations consistent with increasing the BSFL levels into layer diets (Table 6). Furthermore, a linear increase ($p < 0.05$) in the Ab titer against SRBC and a linear and quadratic increase ($p < 0.05$) in the PHA-WST were obtained as the BSFL level into layer diets increased (Table 6).

Table 6: Effect of dietary black soldier fly larvae (BSFL) meal on triiodothyronine, estradiol, anti-sheep red blood cells antibody, and phytohemagglutinin-wattle swelling responses of laying hens.

Dietary BSFL meals	T_3, µmol/mL	E_2, pg/mL	Anti-SRBC Ab, $\log_2$	PHA-WST, mm
0%	5.62^{c}	388.33^{c}	6.56^{c}	0.51^{c}
3%	5.34^{c}	401.11^{c}	6.67^{c}	0.54^{c}
6%	8.15^{b}	427.22^{bc}	8.33^{ab}	0.67^{bc}
9%	9.89^{a}	525.89^{ab}	8.11^{b}	0.87^{b}
12%	10.01^{a}	551.11^{a}	9.22^{a}	1.49^{a}
SEM	0.531	37.504	0.355	0.107
P-value				
Combined	< 0.001	< 0.001	< 0.001	< 0.001
Linear term	< 0.001	< 0.001	< 0.001	< 0.001
Quadratic term	0.847	0.332	0.906	< 0.001

Means within the same column (n = 18 birds per treatment group) presented with uncommon letters differ significantly at p-value < 0.05. T_3, triiodothyronine hormone; E_2, estradiol hormone; Anti-SRBC Ab, anti-sheep red blood cells antibody; PHA-WST, phytohemagglutinin-wattle swelling. SEM = pooled standard error of means.

ECONOMIC EFFICIENCY

Data of Table 7 show the economic efficiency of partially replacing SB and C meals with BSFL meal in laying hens' diets. There was no observed effect of BSFL meal inclusion on the total FI and the total feed cost per bird during the experimental period. In contrast, increasing the dietary BSFL inclusion level into the layer diets linearly increased the total egg number produced, the total revenue, and the profit margin per bird. In addition, a linear ($p < 0.05$) increase in the CBR and RoI outcomes was obtained as the BSFL meal increased in the diets. The EE of BSFL inclusion was linearly ($p < 0.05$) increased as the level of the BSFL increased, recording approximately 2.32 p.p. increment in the 12% BSFL group compared to the control basal diet (0% BSFL).

DISCUSSION

The use of different substrates in feeding the BSFL during their production may affect the contents of the crude protein (ranging 35-49%) and the amino acids in the final BSFL meal (Fuso *et al*., 2021). We used a whole-dried source of larvae with a full-fat composition, and this could explain the low CP (34%) and high fat (40%) contents of this BSFL meal compared to that reported by, e.g., Mwaniki et al. (2018) who applied a defatted source of BSFL in their study (56.1% CP and 6.8% fats). In addition, the SB meal used in the present study showed a higher CP (44%) than that presented in the BSFL meal (Table 2). Thus, around 11%, 22%, 29% and 36% of the SB meal as a common protein component in the control diet was partially substituted with 3%, 6%, 9%, and 12% of BSFL meal, respectively, while the corn and other ingredients were re-formulated to compose the other experimental diets with the same nutritional levels (Table 1). Our target was to investigate the prospective effects of the BSFL meal insertion into the layer diet on their productivity, egg quality, physiological and economic performance.

Some outputs of the hen's performance were improved after the inclusion of the BSFL meals in the diets. The BSFL meal groups of 9% and 12% showed an increase in the EP and EW by at least 3.38 percent points and 1.54 g, respectively, and a decrease in the FCR by at least 20%, compared to the basal diet group without BSFL supplementation. In contrast, the body weights and feed consumption did not differ among the BSFL meal groups (Table 3). These results may practically presume that hens were able to keep their endogenous balances and that they efficiently used the protein available in the BSFL meal without developing any metabolic disorders (Maurer et al., 2016). The increased EP and EW in the BSFL-treated groups may also be attributed to the high fat contents in the BSFL, since a maximizing effect for increasing the fat levels in isoenergetic diets on egg production was previously reported in laying hens and could be attributed to the fat-depending improvement of available metabolizable energy and linoleic fatty acid levels (Mateos et al., 2012).

Furthermore, the features of egg quality were linearly improved in proportional to the higher levels of the BSFL treatment. Indeed, it is possible to suppose that

Table 7: Economic efficiency of partially replacing soybean (SB) and corn (C) meals with BSFL meal in laying hens' diets.

Items	Dietary BSFL meals						P-value		
	0%	3%	6%	9%	12%	SEM	combined	Linear term	Quadratic term
Costs									
Total feed intake (kg/bird)	6.98	7.08	7.04	7.07	6.97	0.053	0.136	0.845	0.022
Basal diet cost (USD/bird)[1]	3.84	3.89	3.87	3.89	3.83	0.029	0.136	0.845	0.022
BSFL meal cost (USD/bird)[2]	0.000^{e}	0.006^{d}	0.012^{c}	0.018^{b}	0.024^{a}	0.000	< 0.001	< 0.001	1.000
Total feed cost (USD/bird)	3.84	3.90	3.88	3.91	3.86	0.029	0.116	0.474	0.022
Benefits									
Total egg number/bird	62.48^{c}	62.37^{c}	64.11^{b}	65.00^{a}	64.81^{a}	0.210	< 0.001	< 0.001	0.079
Total revenue (USD/bird)[3]	13.75^{c}	13.72^{c}	14.10^{b}	14.30^{a}	14.26^{a}	0.046	< 0.001	< 0.001	0.079
Profit margin (USD/bird)[4]	9.91^{c}	9.82^{c}	10.22^{b}	10.39^{a}	10.40^{a}	0.056	< 0.001	< 0.001	0.810
CBR[5]	3.6^{bc}	3.5^{c}	3.6^{ab}	3.7^{ab}	3.7^{a}	0.03	< 0.001	< 0.001	0.170
RoI[6]	258.3^{bc}	252.0^{c}	263.3^{ab}	266.2^{ab}	269.7^{a}	3.04	< 0.001	< 0.001	0.170
RoI*[7]	258.3^{bc}	252.5^{c}	264.4^{ab}	267.9^{a}	272.1^{a}	3.05	< 0.001	< 0.001	0.167
EE[8]	0.00^{e}	0.54^{d}	1.13^{c}	1.70^{b}	2.32^{a}	0.022	< 0.001	< 0.001	0.020

Currency exchange during the study was 1 USD for 3.76 SAR. [1]Cost of the basal diet = 0.55 USD/kg. [2]Cost of the BSFL meal = 0.20 USD/kg. [3]Egg price = 0.22 USD/egg. [4]Profit margin = total revenue – total feed cost. [5]Cost benefit ratio = total revenue/total feed cost. [6]Return on investment when BSFL was included = profit margin/total feed cost×100. [7]Return on investment when BSFL was not included = (total revenue – basal diet cost)/basal diet cost×100. [8]Economic efficiency of BSFL meal inclusion = RoI* - RoI. Means within the same raw ($n = 54$ birds per treatment group) presented with uncommon letters differ significantly at p-value < 0.05. SEM = pooled standard error of means.

the improvement effect of the BSFL treatment on egg production and quality traits may be associated with the enhancement of the physiological status of the laying hens (Sypniewski et al., 2020). Hence, some physiological mechanisms were evaluated in the present study. The plasma TP, TG, CH, and CA were linearly increased by increasing the BSFL meal in the diet. The increase in plasma proteins may promote the formation of egg albumen through unifying with the polysaccharides, polyphenols and β-ovomucin secreted from the hen's oviduct (Ariana et al., 2011), and this in turn may also contribute to the increase of egg HU. The increase in YC intensity may be correlated with the dark color of melanin pigments that existed in the cuticle of the insects (Ushakova et al., 2018). In addition, the BSFL meal increased the egg yolk concentration of pigments, such as γ-tocopherol, lutein, β-carotene, and total carotenoids, in comparison with the SB meal (Secci et al., 2018). As shown in Table 2, the BSFL meal used in the current study contains 3-fold calcium and phosphorus more than the SB meal. Although the level of CA was evenly adjusted in the final experimental diets, substituting the SB meal of layer diets with the increasing levels of the BSFL meal throughout the 10 weeks of this experiment resulted in increasing the plasma CA concentration of laying hens. This result gives a reason to assume that the bioavailability of CA obtained from the BSFL meal is higher than that obtained from the SB meal. Consequently, the improved ST and SS of the egg could be explained by enhanced CA absorption from the BSFL diets by the hen's guts (An et al., 2016). The increase in TG and CH is expected due to using BSFL meal derived from a whole-dried source of larvae with a full-fat composition. Thus, in some cases, such fatted-BSFL could be considered as a valuable source of energy for growing chickens (de Marco et al., 2015; Schiavone et al., 2018). In the present study, the plasma TG and CH were linearly increased by increasing the BSFL meal in the diet (Table 5). The consumption of foods rich in dietary cholesterol may increase the low-density lipoprotein particles in the individual blood (Lewington et al., 2007), which can induce a high incidence of atherosclerotic cardiovascular disease (CVD), especially in old-aged individuals (Weggemans et al., 2001). Therefore, BSFL meal should be used carefully in the layer diets to avoid any possible harmful effects on human health.

The positive effect of BSFL on the egg-laying performance and quality traits suggests that BSFL may have a potential effect on the hormonal secretion from the thyroid and/or ovary glands. The current study revealed a linear increase in the T_3 and E_2 hormones as a response to the increase of the BSFL meal levels as a replacement of the SB meal. Similar results were obtained in T_3 hormone when 50-100% of fish meal diet was replaced by BSFL meal in adult layer chickens (Attivi et al., 2022). The increase of T_3 concentration in our study may be due to the higher concentration of tyrosine amino acid, which is the precursor of thyroid hormones, in the BSFL meal than that in the SB meal (Table 2) (Khaliq et al., 2015). In contrast, the BSFL meal increased the plasma CH, which is the precursor of all steroid hormones including the E_2 (Manley and Mayer, 2013). In addition, the increase of E_2 could be referred to the functional regulation of T_3 hormone itself for the synthesis and secretion of steroid hormones from the female reproductive system (Silva et al., 2018). In agreement with our results, Hatab et al. (2020) elaborated that partial or total replacement of meat-bone meal in growing quail diet with insect meal derived from cotton leafworm larvae substantially increased the serum levels of TP, TG, CH, total antioxidants, thyroxin, E_2, and testosterone. On the other hand, the high levels of E_2 in the BSFL-treated hens may contribute to the other physiological mechanisms related to egg formation and quality (Mishra et al., 2019), such as the synthesis of egg albumen and yolk precursors and the regulation of calcium metabolism for the eggshell thickness and strength.

The anti-SRBC-Ab titer and the PHA-WST were also evaluated as indicators for the humoral and the cellular immune responses, respectively, to the BSFL treatment in this study. The results showed a significant increase in both parameters by increasing the level of BSFL meal in the layer diet (Table 6). This positive effect on immunity has been attributed to the presence of appreciable levels of chitin and antimicrobial peptides in addition to lauric fatty acid in the whole meal of insects or their larvae without defatting (Gasco et al., 2018; Koutsos et al., 2022). Immune stimulation by using insect products as functional feed additives has been frequently demonstrated in various animals, including broilers (Lee et al., 2018), Turkey (Sypniewski et al., 2020), swine (Spranghers et al., 2018; Yu et al., 2020), dogs (Lei et al., 2019), and aquatic animals (Gopalakannan and Arul, 2006; Vahedi and Ghodratizadeh, 2011; Weththasinghe et al., 2021). However, more data are needed to characterize the prospective role of BSFL meal insertion in poultry diets on improving their immunity and health, especially when confronting challenges.

In the present study, the EP, EW, FCR, and all egg quality traits seem to be improved in a linear response to the increase of dietary BSFL meal level up to 12% in the diet (36% substitution of SB meal). Referring to previous studies, Marono et al. (2017) found that total replacement of SB with BSFL meal negatively affected the performance of hens, while Agunbiade et al. (2007) suggested that higher substitution of more than 50% of fish meal with maggot meals (collected and processed from poultry waste) showed adverse effects on egg production and shell strength. In another study on 45-wk-old laying hens, Liu et al. (2021) observed that BSFL meal should be added to the diet for a duration of more than 3 weeks to avoid the negative impacts of BSFL on the egg traits at the early stage of supplementation. These data assume that the effects of BSFL meal inclusion into layer diets can be accredited by various factors, such as the age and line of the birds, and the stage, the dose, and the duration of BSFL treatment (Liu et al., 2021; Elahi et al., 2022).

The economic study showed that the total cost of the diets did not statistically differ and were not influenced by the increase in the cost of BSFL meal replacing the SB meal (Table 7). However, other studies reported that nutritional expenses can be reduced by utilizing insects in poultry diets (Khan et al., 2016; Onsongo et al., 2018). The profit margin was increased by 3% when using 6% BSFL meal while it was increased by 5% when using 9-12% BSFL meal, compared to the basal diet. The addition of 12% BSFL achieved the highest CBR (3.70) and RoI (269.75) compared to the other diets. Our results indicated that 12% BSFL treatment group showed the best outcomes recording a 2.32 p.p. increase in the economic efficiency. Compared to the basal diet group (0% BSFL), SB meal was reduced by 36%. Therefore, it could be suggested, under the conditions of this study, that every 3% of SB meal (containing 44% protein) can be substituted with 1% of BSFL meal (containing 34% protein) to obtain the maximum performance of laying hens.

CONCLUSIONS AND RECOMMENDATIONS

The study concludes that BSFL meal could be partially included into the layer diets as a substitute for the SB meal to enhance egg production, egg quality traits, physiological aspects, and economic efficiency of laying hens. There are linear and quadratic impacts for increasing the levels of BSFL meal into the layer diets on most of the studied parameters. However, the highest economic outputs were obtained with the use of 12% BSFL (120 g/kg of the layer diets). Moreover, our results indicated that each 3% of SB meal can be subrogated by 1% BSFL meal in the commercial layer diets, to achieve a favorable performance and high economic outputs of egg production.

ACKNOWLEDGMENTS

The authors would like to thank the Deanship of Scientific Research and Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (grant number: GRANT855) for the financial support provided to conduct and publish the discussed research.

NOVELTY STATEMENT

The present investigation is one of the first to study the effect of effect of black soldier fly larvae (BSFL) inclusion as a main ingredient instead of soybean-corn meals into diets of laying hens on their productive performance, egg traits, physiological aspects, and economic efficiency.

AUTHOR’S CONTRIBUTION

All authors contributed equally to the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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