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Effects of Supplementation Black Soldier Fly Larvae (*Hermetia illucens* L.) to The Diets of Breeder Japanese Quails on Performance, Egg Quality, and Incubation Parameters

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HIGHLIGHTS

- The nutritional composition of the black soldier fly, one of the edible insects, is quite rich.
- Black soldier fly has a high potential as an alternative feed source.
- In the experiment, the effects of black soldier fly larvae used in breeder quail diets were determined.

Abstract

This study was performed to assess the effect of different levels of addition (0, 4, and 8%) of dried and ground Black Soldier Fly Larvae *(Hermetia illucens* L.) to breeder Japanese quail (Coturnix coturnix japonica) diets on performance, egg quality and incubation parameters. In the experiment, 72 breeder Japanese quails (18 males, 54 females) at 8 weeks of age were randomly distributed into 3 treatment groups with 6 replications, each with 3 females and 1 male quail. Treatment groups were fed with diets supplemented with different levels of black soldier fly larvae (BSF) for 8 weeks.Feed intake was significantly higher in the control group than in the other treatment groups, and there were no significant differences between the groups in feed conversion ratio, egg production, egg weight, and egg mass. Similarly, no significant differences were observed among the groups in terms of egg quality parameters such as eggshell thickness, eggyolk index, eggshell breaking strength, and eggshell weight parameters. However, the shape index was significantly higher in the BSF 4% group than the control group. Albumen index was highest in the control group. While there was no significant difference among the groups in terms of egg yolk L* value, as the amount of BSF in the diet increased, there was a significant increase in the a* and b* values. Incubation parameters were not affected by the treatments. According to the results of the experiment, it was concluded that the addition of 4% and 8% BSF to breeder Japanese quail diets can be used at 8% level without any negative effects on performance, egg quality, or incubation parameters.

Keywords: Breeder quail, Black soldier Fly (*Hermetia illucens L.*) larvae, egg quality, incubation parameters, performance

1. Introduction

The Food and Agriculture Organization of the United Nations has recognized the potential of using edible insects for food and feed since 2003 and supports the production and sharing of knowledge through

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publications (Mishyna et al. 2020). Insects have the potential to be a possible alternative food source that can help meet the increasing demand and prices of traditional feed raw materials needed in the livestock industry in a more sustainable way(Cullere et al. 2016). Insects are rich in fat, amino acids, carbohydrates, amino acids ,trace elements, and vitamins(Skotnicka et al. 2021). In addition to meeting the required amino acid requirements due to the nutritional content they contain; most insects are also sufficient in terms of energy and protein values, and also rich in fatty acids. Trace elements (such as Cu, Fe, Mg, Mn, P, Se, and Zn) and vitamin contents such as riboflavin, pantothenic acid, biotin, and folic acid are also very high (Rumpold and Schlüter 2013). Oil is found in various forms in insects. Triacylglycerol makes up approximately 80% of the oil present in insects. Phospholipids are the second most important group and their proportion in insect oil is generally less than 20% (Kouřimská and Adámková 2016). Black soldier fly is rich in lauric acid and has been reported to be a suitable lipid source in poultry diets (Schiavone et al. 2017; Kierończyk et al. 2020). The amino acid profile of black soldier fly is similar to fishmeal (Barroso et al. 2014). This study was carried out to determine the effects of dried Black Soldier Fly larvae (BSF) on egg quality, performance, and incubation parameters of 4% and 8% supplementation to breeder quail diets grown with high yield expectation. The suitability of BSF as a feed raw material with high energy and protein values, especially rich in essential amino acids, which can positively affect the yield of animal products was investigated.

2. Materials and Methods

The trial was carried out at the Quail Unit of the Department of Animal Science, Faculty of Agriculture, Selcuk University (Konya, Turkey). The animal experiment was conducted according to the guidelines of the local ethics committee of Selçuk University that were arranged according to the "European Union 2010/63/EU is the European Union (EU) legislation." All processes in this experiment agree with the ethical rules of animal welfare. A total of 72 breeding Japanese quails (54 females and 18 males) at 8 weeks of age were used in the experiment. Quails were randomly allocated to 3 treatment groups with 18 subgroups of 3 females and 1 male each. Breeder quail feed was prepared to contain 2900 Kcal/kg ME and 20% crude protein during the eight-week experiment (NRCCouncil 1994). In the experiment, the basal diet (0%) was prepared based on corn and soybean meal containing dried and ground Black Soldier Fly larvae (BSF;*Hermetia illucens* L.) at 4% and 8% levels (Table 1).

Table 1. Nutrient content of experimental diets

Ingredients	Black Soldier Fly Larvae Meal %		
	0	4	8
Corn	50.1	52.60	54.60
Black soldier fly larvae meal	0	4.0	8.0
Soybean meal	36	31.2	27.00
Soyoil	6.2	4.5	2.7
Limestone	5.10	5.10	5.10
Dicalcium phosphate(DCP)	1.8	1.8	1.80
Salt	0.25	0.25	0.25
Premix[1]	0.25	0.25	0.25
L-Lysine	0.10	0.10	0.10
DL-Methionine	0.20	0.20	0.20
Calculated nutrients			
Crude protein, %	20.00	20.03	20.29
Metabolizable Energy, kcal/kg	2904	2922	2929
Calcium, %	2.50	2.49	2.48
Available phosphorus, %	0.35	0.35	0.34
Lysine, %	1.04	0.94	0.89
Methionine, %	0.47	0.45	0.44
Methionine-cysteine, %	0.70	0.70	0.70

[1]: Premix provided the following per kg of diet: vitamin D_3: 2.200 IU, vitamin E: 11 mg, vitamin A: 8.800 IU, nicotinic acid: 44 mg, Cal-D-pantothenate: 8.8 mg, thiamine: 2.5 mg, vitamin B_{12}: 6.6 mg, riboflavin: 4.4 mg, folic acid: 1 mg, D-biotin: 0.11 mg, choline: 220 mg, iron: 60 mg, zinc: 60 mg, manganese: 80 mg, copper: 5 mg, selenium: 0.15 mg, cobalt: 0.20 mg, iodine: 1 mg, BSF: dried black soldier fly larvae

Dried and ground black soldier fly larvae purchased from a commercial company were used.The duration of the experiment was 8 weeks. A lighting program of 16 hours light / 8 hours dark was applied in the experiment. Feed and water were given ad-libitum. Body weight (BW) of quails were determined at the beginning and the end of the experiment by weighing the quails in a group. Egg production (EP) and feed intake (FI) were recorded daily. Egg weight (EW) was calculated by averaging the weights of all eggs collected in each subgroup during the last 2 days of each four-week period. Egg mass (EM) was calculated using the formula egg production×egg weight/period. Feed conversion ratio (FCR) was calculated using the ratio of feed intake to egg mass. Eggshell breaking strength, shell thickness, shell weight, egg weight, shape index, yolk and albumen index, Haugh unit were determined in the eggs collected on the last 2 days of each 4-week period during the experiment. Measurements were made twice and the results are given as the average of these two measurements. Egg shell weight (%) was calculated by the formula egg shell weight (g)/egg weight x 100. Egg shell breaking strength was measured by applying pressure to the blunt part of the egg with an assisted system (Egg Force Reader, Orka Food Technology, Israel). Egg shell thickness was calculated by averaging the numbers obtained from three points of the egg using a micro meter (Mitutoyo, 0.01 mm, Japan). Egg yolk color was measured as L*, a* and b* values by colorimeter (KonicaMinolta CR410).

At the end of the experiment, the eggs collected for 7 days were placed in the incubator with incubation parameters. After the seventeenth day, the number of hatched chicks and after the twentieth day, the number of fertilized and unfertilized eggs were determined by breaking the unhatched eggs. Hatching efficiency, fertility rate and hatchability were calculated according to the data obtained(Erensayın 2000). Statistical analysis of the data obtained from the experiment was performed according to one-way analysis of variance (ANOVA) (Minitab 2000) and Duncan multiple comparisons test was used to determine the differences between the means.

3. Results

The results of the effects of dried and ground BSF at 0%, 4% and 8% levels on BWC, FI, FCR, EP and EM parameters of broiler Japanese quail diets are given in Table 2.

Table 2. The effect of dietary different levels of BSF on the performance parameters of breeder Japanese quails

Parameters	Control	BSF **4 %**	BSF **8 %**	P-Value
Initial body weight (g)	212.17 ± 3.34	208.71 ± 4.10	204.92 ± 4.43	0.456
Final body weight (g)	228.39 ± 2.76	226.50 ± 3.80	223.85 ± 4.53	0.699
Body weight change(g)	16.222 ± 3.16	17.792 ± 4.41	18.931 ± 3.38	0.874
Feed intake (g)	26.55 ± 0.32^{A}	24.90 ± 0.31^{B}	24.89 ± 0.31^{B}	0.003
Feed conversion ratio	2.64 ± 0.07	2.45 ± 0.05	2.45 ± 0.05	0.073
Egg production (%)	85.21 ± 1.48	83.53 ± 0.71	83.43 ± 0.49	0.382
Egg weight(g)	11.818 ± 0.288	12.179 ± 0.268	12.162 ± 0.209	0.548
Egg mass(g/quail/day)	10.06 ± 0.22	10.17 ± 0.26	10.14 ± 0.18	0.932

A,B: The differences indicated by different letters on the same row are statistically significant, P<0.01

The effect of different levels of BSF supplementation to the diet on BWC, FCR, EP, EW and EM parameters was not statistically significant (P>0.05). The highest feed intake was in the control group. Feed intake in the control group was significantly higher than BSF 4% and BSF 8% groups (P<0.01). There are studies reporting that the addition of BSF in various forms (live, larvae, etc.) to broiler diets resulted in higher feed intake in the control group compared to the treatment groups(Murawska *et al.* 2021; Mat *et al.* 2022). However, there are studies with laying hens that report that feed intake is not affected(Maurer *et al.* 2016; Marono *et al.* 2017; Bellezza Oddon *et al.* 2021; Elangovan *et al.* 2021; Yan *et al.* 2023). In the experiment in which the effects of black soldier fly maggot substitution instead of fish meal in laying quails were investigated, it was reported that feed intake decreased in the treatment groups compared to the control group, but there was no significant effect on egg production(Widjastuti *et al.* 2014). Addition of defatted black soldier fly larvae meal up to 15% to laying quail diets did not cause any negative effects and it was concluded that it could be a potential substitute for soybean meal. No negative effects on hatching performance and egg quality were observed(Dalle Zotte *et al.* 2019). It has been reported that defatted black soldier fly (*Hermetia illucens*) larval meal can be used as a protein source in laying hens without adverse effects on animal health and has positive effects on immunity(Marono *et al.* 2017). In the present study, the addition of dried BSF larvae at 4% and 8% levels to breeder Japanese quail diets had no negative effect on performance. The effects of addition of dried and ground BSF to breeder Japanese quail diets on egg quality parameters, yolk L*, a* and b* values are given in Table 3. The effect of BSF addition to breeder Japanese quail diets on egg shell breaking strength, shell thickness, shell weight, shell ratio, yolk index, Haugh Unit and L* values were not statistically significant (P>0.05). In terms of shape index, the statistical difference between BSF 4% group and BSF 8% group was not significant, while the difference between BSF 4% group and control group was significant (P<0.05). The difference between BSF 8% group and control group was insignificant (P>0.05). While the difference in albumen index between the control and BSF 8% group was insignificant (P>0.05), the difference between the control and BSF 4% group was significant (P<0.05). The difference between BSF 4% and BSF 8% groups was also insignificant (P>0.05). There was an increase in a* value with increasing BSF in the diet. The difference between BSF 8% group and BSF 4% group in a* value between the treatment groups was insignificant, while the difference between BSF 8% group and control group was significant (P<0.05). The difference between the BSF 4% group and the control group was insignificant (P>0.05). The highest b* value was measured in BSF 8% group. Egg yolk b* value showed similar results to a*, and b* increased in direct proportion to the increasing amount of BSF in the diet.

Table 3. The effect of dietary different levels of BSF on the egg quality, yolk L*, a* and b* values of breeder Japanese quails

	Control	BSF 4 %	BSF 8 %	P-Value
Eggshell breaking strength (N)	11.720 ± 0.514	12.133 ± 0.727	12.962 ± 0.543	0.357
Eggshell thickness (mm)	0.229 ± 0.008	0.221 ± 0.002	0.214 ± 0.001	0.135
Eggshell weight (g)	1.116 ± 0.084	1.103 ± 0.031	1.144 ± 0.017	0.856
Eggshell ratio (%)	9,453 ± 0,579	9,111 ± 0,274	9,472 ± 0,298	0,783
Egg shape index (%)	75.611 ± 0.328 [b]	77.337 ± 0.378 [a]	76.658 ± 0.571[ab]	0.042
Albumen index (%)	6.3208 ± 0.163 [a]	5.6983 ± 0.102 [b]	6.0176 ± 0.130 [ab]	0.017
Eggyolk index (%)	47.052 ± 1.02	44.550 ± 1.59	48.430 ± 0.334	0.072
Haugh Unit	92.642 ± 0.649	90.546 ± 0.263	92.165 ± 0.799	0.069
L*	41.845 ± 0.83	42.905 ± 0.49	43.732 ± 0.78	0.211
a*	5.827 ± 0.08[b]	6.1700 ± 0.27[ab]	6.555 ± 0.13[a]	0.039
b*	21.324 ± 0.79[b]	23.079 ± 0.53[ab]	24.641 ± 0.55[a]	0.029

[A,B]: The differences indicated by different letters on the same line are statistically significant, P<0,01

[a,b]: The differences indicated by different letters on the same row are statistically significant, P<0.05

Hopley (2016) reported that the addition of BSF larvae and prepupal BSF to laying hen did not affect egg albumen height and albumen weight, yolk L*, significantly increased the a* value and increased the b* value numerically but not statistically significant. Addition of black soldier fly to laying hen diets has been reported to result in brighter egg yolk compared to the control group(Al-Qazzaz *et al.* 2016). It was shown that the addition of BSF larvae instead of fish meal to laying quail diets had no significant effect on egg weight, Haugh unit, yolk index and yolk color, but it significantly affected egg production and eggshell thickness(Purwanti and Nahariah 2020). Finke (2013) stated that black soldier fly larvae contain the carotenoids beta-carotene, lutein and zeaxanthin and that these carotenoids are generally responsible for the yolk pigmentation in feeds. This may be the possible reason for the increase in egg yolk a* and b* value.

The results of the effects of dried and ground black soldier fly larvae at 4% and 8% levels on the incubation parameters of broiler Japanese quail diets are given in Table 4.

Table 4. The effect of dietary different levels of BSF on incubation parameters of breeder Japanese quails

	Control	BSF 4 %	BSF 8 %	P-Value
Hatching weight (g)	7.93 ± 0.163	8.24 ± 0.116	8.35 ± 0.235	0.259
Hatchability (% of fertile eggs)	98.61 ± 1.39	97.10 ± 1.84	98.61 ± 1.39	0.733
Fertility (%)	98.61 ± 1.39	98.61 ± 1.39	97.22 ± 1.76	0.761
Hatchability(% of setting eggs)	97.22 ± 1.76	95.83 ± 2.85	95.83 ± 1.86	0.878

The treatment groups in terms of incubation parameters such as chick's weight, hatchability and fertility were found to be insignificant (P>0.05). It was reported that the addition of black soldier fly larvae to laying hen diets instead of 5% of soybean meal did not affect fertility and hatchability(Petkov *et al.* 2022). In this study, the addition of black soldier fly larvae to the diet did not have any negative effect on incubation parameters

4. Conclusions

In conclusion, the supplement of BSF meal to the diets of breeder Japanese quail did not have any detrimental impact on performance, egg quality and incubation parameters. There is a need for research on the use of black soldier fly, which has a high potential as an alternative feed source, in animal nutrition. As a result of the experiment, the use of dried and ground BSF meal 8% level in breeder Japanese quail diets can be recommended.

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