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Does Mealworm (*Tenebrio molitor*) Can be Considered as a Functional Additive in Japanese Quails̉ Diets?

Research Article

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ABSTRACT

This study was conducted to evaluate the effects of mealworm (*Tenebrio molitor*) dietary supplementation on egg production, egg traits, humoral immunity and ileal microbiota in Japanese quails. A hundred and twenty female Japanese quails (*Coturnix coturnix japonica*) were used in a completely randomized design with three treatments, five replicates, and eight birds in each replicate. Experimental diets included: basal diet (without any additives), basal diet + 0.1% full-fat mealworm powder, basal diet + 0.2% mealworm powder. The experiment lasted five weeks, and the quails had free access to the feed and water. The results showed that mealworm supplementation did not have any significant effect on feed conversion ratio of the quails during the whole period of the experiment. At the 5th week of the experiment, egg weight and egg mass of the quails fed with mealworm increased linearly, and the feed intake of the birds fed with 0.1% mealworm was different quadratically. Different levels of mealworm powder supplementation increased egg albumen weight and yolk height linearly during the whole period of the experiment. Adding mealworm powder increased antibody titer against sheep red blood cell in laying quails on 89 and 96 d of age. It is concluded that mealworm supplementation at the level of 0.1% improved egg weight, egg production, albumen weight, yolk height, and humoral immunity of Japanese laying quails, so it has the potential to be considered as an organic functional additive in quails' diet.

KEY WORDS humoral immunity, Japanese quails, mealworm.

INTRODUCTION

Using insects in poultry diet as a protein supplement is a way that can help to reduce the feed cost (Khusro *et al.* 2012), because agricultural by-products and wastes can be used for rearing insects that have high nutritional value (Bava *et al.* 2019). It is well known that chicken eat insects during all periods of their life when rearing in an outdoor system (Bovera *et al.* 2015). In fact, insects are natural source of protein for the birds in the wildlife (Al-Qazzaz *et al.* 2016), and many researchers have applied insects as a substitute for soybean meal or fish meal in commercial poultry feed without any negative effect on the birds' performance; in laying hen (Agunbiade *et al.* 2007; Amao *et al.* 2010; Al-Qazzaz *et al.* 2016; Maurer *et al.* 2016), in broiler chicken (Bovera *et al.* 2015; Khan *et al.* 2018; Kierończyk *et al.* 2018; Velten *et al.* 2018; Dillak *et al.* 2019) and in meat quails (Zadeh *et al.* 2019). Also, the FAO reported that using insects in human food and animal feed is a good way for decreasing penury (FAO, 2010). Rumpold and Oliver (2013) found that insects have different orders such as Diptera (black soldier fly, housefly), Coleoptera (mealworms), Megadrilacea (earthworm), Lepidoptera (silkworm and *Cirina forda*) and Orthoptera (grasshoppers, locust and crickets). Regards to different insect species, gender, their life stage, diet, and the environment, it was reported that insects contain 38 to 76% crude protein, and 14 to 43% crude fat (Józefiak *et al*. 2016a; Józefiak *et al*. 2018). Besides, some insect species contain bioactive antimicrobial or antifungal peptides or polypeptides that may help to overcome critical challenges such as antibiotic-resistant infection (Manniello *et al*. 2021). *Tenebrio molitor* is one of the insects that can be mass-reared due to its ability to use several substances as feed ingredients (Chia, 2019). Linnaeus was the first researcher who described *Tenebrio molitor* in 1758. Bovera *et al*. (2016) reported that using *Tenebrio molitor* in broilers' diet improved the feed conversion ratio (FCR) and immune response of the birds. It was reported that using 5% black soldier fly larvae in the diet of laying hens improved their egg production, but the birds' feed intake was not changed by insect consumption (Al-Qazzaz *et al*. 2016). Józefiak *et al*. (2018) reported that 0.2% supplementation of *T. molitor* and *H. illucens* increased feed intake (FI) of broiler chickens. In another experiment, Józefiak *et al*. (2018) found that adding 0.2% to broilers' diets of *S. lateralis* improved the birds' body weight gain, feed intake (FI) and FCR. This study evaluated the effects of mealworm (*Tenebrio molitor*) supplementation in small amounts on egg production, egg traits, humoral immunity and ileal microbiota in laying *Japanese* quails.

MATERIALS AND METHODS

Birds and housing

This experiment was conducted in January 2020. A total of 120 female Japanese quails (*Coturnix coturnix japonica*, 60 days old) were purchased from a local quail farm. The birds´ average body weight was 240 ± 2 g, and they randomly allocated to three treatments with five replicates, each consisting of 8 birds. Each replicate was done in a cage (0.4×0.9 m). The quails were reared following the guidelines of the Institutional Animal Care and Ethics Committee of the Iranian Council of Animal Care (Care ICoA, 1995). The rearing house temperature and relative humidity were kept at 22 ± 1 ˚C, and 50-60%, respectively. The quails were reared under a lighting program of 16 h/d light cycle with an average light intensity of 40 lux/m^2. The quails did not receive any vaccine during their rearing and production periods.

Diets and feeding program

The feed ingredients were analyzed for crude protein (CP), crude fat (CF), crude fiber (CF) starch and total sugar according to AOAC (2005) methods. Metabolizable energy (MEn) of the main ingredients was calculated based on feedstuffs' analyzed values (NRC, 1994).

Then, the basal diet (Table 1) was formulated using WUFFDA software according to nutritional requirements of laying Japanese quails described in NRC (1994). After preparing the basal diet, crude protein (CP), crude fat (CF), crude fiber (CF) of the diet was evaluated as regards AOAC (2005) procedures. The birds were raised in accordance with the guidelines of the Institutional Animal Care and Ethics Committee of the Iranian Council of Animal Care (Care ICoA, 1995).

The mealworm (*Tenebrio molitor*) powder was supplemented on top of the basal diet. The dietary treatments were: 1. control (basal diet without any additive), 2. basal diet + 0.1% mealworm powder, 3. basal diet + 0.2% mealworm powder. The birds have free access to water and feed in mesh form with a 2 mm mean particle size. Two weeks was considered as an adaptation period for the quails. Quails fed with experimental diets during the whole period of the study (days 61-96 of age).

Preparation of mealworm

Tenebrio molitor larvae was purchased from a commercial producer, air-dried, and grounded. Before using in the experiment, the proximal analysis of the mealworm powder was evaluated according to AOAC (2005). The crude protein (CP), ether extracts (EE), crude fibre (CF), ash of *Tenebrio molitor* larvae was 45.01 ± 0.34, 30.75 ± 0.34, 4.63 ± 0.20, 3.07 ± 0.05 percent (as dry matter), respectively.

Egg production performance

The average FI of the birds was calculated weekly by subtracting the left-over feed from the quantity supplied to the quails in each cage per week. Every day, the eggs from each replicate were weighed, and then the average egg weight was calculated weekly. The FCR was calculated by dividing the FI to egg mass (average egg number × average egg weight) in each pen and adjusted for mortality. The hen day egg production was calculated by the following formula: egg production= number of egg production on each day / number of hens alive on that day × 100 (North and Bell, 1990).

Egg quality traits

The average egg weight, egg length, egg width, albumen weight, yolk weight, eggshell weight, shell thickness, albumen height, yolk diameter and yolk height were measured daily. Briefly, eggs from each replicate were weighed individually and broken in a plate to evaluate their quality. The egg shape index and yolk index were calculated as: egg shape index (%)= [width (cm) / height (cm)] × 100, yolk index= yolk height / yolk diameter, respectively. The Haugh unit was calculated with the formula according to Kul and Seker (2004): HU= 100 log (albumen height (mm)+7.57–1.7 W0.37).

Table 1 Ingredients and nutrient composition of the basal diet[1]

Ingredients (%)	61-96 days
Yellow corn	51.31
Soybean meal (44%)	36.70
Vegetable oil	4.00
Wheat barn	0.20
Oyster shell	5.64
Dicalcium phosphate	1.17
Common salt (NaCl)	0.35
DL-methionine	0.13
Vitamin and mineral premix[2]	0.5
Calculated contents (%)	
Metabolizable energy (ME) (kcal/kg DM)	2910
Crude protein	20.03
Calcium	2.52
Available phosphorus	0.36
Sodium	0.15
Methionine	0.45
Lysine	1.12
Methionine + cystine	0.78
Threonine	0.78
Analyses contents (%)	
Dry matter (DM)	90.13
Crude protein	19.92
Crud fat	5.58
Crude fiber	3.74

[1] Control group was fed the basal diet. The other groups fed the same basal diet supplemented with mealworm powder at the levels of 0.1 or 0.2 %.
[2] Vitamin and mineral premix supplied the followings per kilogram of diet: vitamin A (retinyl acetate): 150 µg; vitamin D_3 (cholecalciferol): 1250 µg; vitamin E: 50 mg; vitamin K_3: 3 mg; Thiamin: 4 mg; vitamin B_{12}: 2 mg; Pantothenate: 50 mg; Folic acid: 1 mg; Riboflavin: 8 mg; Biotin: 200 µg; Choline chloride: 500 mg; Mn: 55 mg; Se: 127 mg; I: 484 mg; Cu: 10 mg; Fe: 80 mg; Zn: 80 mg and KI: 1 mg.

Humoral immunity response

To evaluate the immune response of the laying quails, sheep red blood cell (SRBC) was used as T-dependent antigens to study the antibody response. On days 82 and 89, six quails from each replicate were injected with 0.2 mL SRBC 5% per bird intramuscularly and blood samples were collected 7 d after the first and second injections from the wing vein of the birds. The serum of blood samples was separated, heat-inactivated at 56 ˚C for 30 min, and then analyzed for total antibody by microhemagglutination assay technique. The antibody titers expressed as the $\log_2$ of the highest dilution of serum that agglutinated an equal volume of 0.5% red blood cells (Grasman, 2010).

Ileal microbiota

At the end of the experiment (96 d of age), six quails of each pen (replicate) with the weight near to average weight of the quails in that cage were killed by cervical dislocation. Then, the ileal content (from Meckel's diverticulum to ileocecal junction) was quickly collected into sterile plastic containers and frozen at -80 ˚C to determine *Escherichia coli*, and *Lactobacillus* counts according to Hu *et al.* (2012). Two mL sterilized saline were used to dilute 0.2 g of ileal content, then 10-fold serial dilutions (10^{-4}, 10^{-5} and 10^{-6}) were prepared.

A 100 µL of the dilutions was transferred onto sterile plates. *Lactobacillus* count was assessed on De-Man, Rogosa and Sharpe (MRS) agar at 37 ˚C after 48 h, and *E. coli* O157:H7 colonies were measured on MacConkey agar at 37 ˚C after 24 h. The bacterial count expressed as 1 g colony forming units (CFU) per gram of ileal content.

Statistical analysis

This study was done in a completely randomized design, and the data were analyzed by analysis of variance using General Linear method (GLM) procedures (SAS, 2001). The data were tested for normal distributions using JMP® software (version 14). All percentage data were subjected to arc sine trans-formation prior to analysis. Conclusions were drawn from the transformed data, only untransformed data are presented for relevance. The mean differences assessed by Duncan's multiple range test at $P \leq 0.05$ (Duncan, 1955). Each cage considered as an experimental unit for evaluating the quails' laying performance and egg traits, and a group of six birds from each cage examined for humoral immunity and ileal microbiota. The model of the present study was as bellow:

$$Y_i = \mu + T_i + \delta_{ij}$$

Where:
Y_i: observed dependent variable.
μ: overall mean.
T_i: effect of mealworm supplementation level.
δ_{ij}: random error.

RESULTS AND DISCUSSION

The results showed that mealworm supplementation did not have a significant effect on egg weight, egg mass, FI, FCR, and egg production of the quails at the first week of the experiment (P>0.05, Table 2). Adding 0.2% mealworm powder decreased egg production by about 8.5 percentage during the second week (P=0.003). Also, the FCR of the quails fed with 0.2% mealworm increased linearly (P≤0.01) during the second week of the experiment. However, the FCR decreased linearly (P≤0.05) with increasing dietary mealworm level at the third week. In the 4^{th} week of the investigation, there was no significant difference among the egg weight, egg mass, FI, FCR, and egg production of the quails (P>0.05). At the 5^{th} week of the experiment, the egg weight and the gg mass of the quails increased linearly (P≤0.05), and the FI of the birds were different quadratically (P≤0.05). During the whole, the egg weight of the quails-fed mealworm up to 0.2% increased linearly (P≤0.05). Interestingly, adding 0.1% mealworm powder improved egg production during the whole period of the experiment (P≤0.05).

Regards to Table 3, mealworm powder supplementation increased egg albumen weight and yolk height linearly during the whole of the experiment (P≤0.05). Mealworm supplementation did not have any significant effect on egg length, egg width, yolk diameter, yolk weight, albumen height, yolk index, shape index, and Haugh unit (P>0.05). It is interesting to note that adding mealworm powder to quails' diet increased Haugh unit numerically.

Adding different levels of mealworm powder to laying Japanese quails' diet improved their humoral immunity response linearly (P≤0.05) as increased antibody titer against SRBC on 89 and 96 d of age (Table 4).

Mealworm supplementation did not have any significant effect on the ileal *Lactobacillus* and *Escherichia coli* population in laying Japanese quails on the last day of the experiment (P>0.05, Figure 1).

It was reported that insect meal has the potential of technically usage in laying quails' diet, and provide optimal performance and health status (Dalle Zotte *et al.* 2019). However, in the present study, adding 0.2% mealworm had negative effect on the FCR of laying quails until the second week of the experiment. This negative effect is may be due to the high content of chitin that causes alteration in intestinal microbiota balance, physiological stress, and poor feed utilization in quails. In a study conducted by Amao *et al.* (2010), it was observed that the replacement of 100 g fish meal with 100 g larvae meal increased the FCR of laying hens. In the present study, during the whole period of the experiment, the egg weight of the quails fed with mealworm up to 0.2% increased linearly. This effect could be due to the nutritional content of insect meal, which is highly nutritive (Sayed *et al.* 2019). Also, adding 0.1% mealworm powder improved egg production of the quails. This effect may relate to higher levels of blood calcium after feeding worm to quails (Hesami *et al.* 2020) and this need more investigation to reach to a comprehensive conclusion. However, mealworm supplementation did not have a significant effect on egg mass, FI, and FCR of the quails during the whole period of the study. Our results are in line with the findings of previous researchers (Dankwa *et al.* 2002; Agunbiade *et al.* 2007; Al-Qazzaz *et al.* 2016; Maurer *et al.* 2016). Dankwa *et al.* (2002) reported that maggot supplementation (30-50 g) improved egg weight (43.5 *vs.* 33.6 g) compared to the control group. Agunbiade *et al.* (2007) used Maggot meal as a fish meal replacement in the diets of old laying hens. Similar to our findings, Agunbiade *et al.* (2007) reported that FI and FCR of laying hens was not changed by using insect in the laying hens' diet. However, hen day egg production was influenced by using a combination of 3.00% fish meal and 4.72% larvae meal.

Maurer *et al.* (2016) applied 12 and 24% defatted black soldier fly larvae meal in laying hens' diet. They reported that there were no significant differences in FI, egg weight and feed efficiency, yolk and shell weights of different experimental groups. Marareni *et al.* (2020) found that using mopane worm (*Imbrasia belina*) up to 150 g/kg in Jumbo quails' diet had no significant effect on feed intake, physiological responses and carcass trait. These results showed that higher levels of insect should be used in the poultry diet to see their significant effect on the performance of the birds. In this study, insect powder supplementation increased egg albumen weight and yolk height, but it did not have any significant effect on the egg length, egg width, yolk diameter, yolk weight, albumen height, yolk index, shape index, and Haugh unit. It seems that the level of mealworm supplementation in our study was not sufficient to change the egg quality in quails. Similar to our results, Agunbiade *et al.* (2007) reported that adding larvae meal had no significant effect on egg quality of laying hens. Also, Ullah *et al.* (2017) used different levels of silkworm pupae (0, 25, 50, 75, 100%) in a 52-week-old laying hens' diet. They found that body weigh (BW), daily FI, hen day production, average egg weight, FCR, and egg quality traits did not change by using different levels of insect in the birds' diet.

Table 2 Effects of mealworm (*Tenebrio Molitor*) on egg production performance of laying quails (*Coturnix coturnix Japonica*) during the whole experiment

First week	**Egg weight (g)**	**Egg mass (g)**	**Feed intake**	**FCR**	**Egg production (%)**
Basal diet	12.068	559.37	38.11	3.816	79.66
Basal diet + 0.1% mealworm powder	12.546	569.76	37.79	3.712	82.74
Basal diet + 0.2% mealworm powder	12.588	529.49	37.96	4.065	75.00
SEM	0.216	22.13	1.087	0.168	2.526
P-value	0.210	0.435	0.978	0.125	0.115
Linear	0.115	0.358	0.922	0.187	0.461
Quadratic	0.427	0.368	0.857	0.163	0.390
Second week	**Egg weight (g)**	**Egg mass (g)**	**Feed intake**	**FCR**	**Egg production (%)**
				**	**
Basal diet	12.156	566.83^{ab}	38.06	3.771^{b}	82.10^{a}
Basal diet + 0.1% mealworm powder	12.372	586.51^{a}	39.28	3.758^{b}	84.64^{a}
Basal diet + 0.2% mealworm powder	12.470	523.83^{b}	38.82	4.158^{a}	75.00^{b}
SEM	0.1217	14.485	0.327	0.088	1.668
P-value	0.216	0.027	0.061	0.011	0.003
Linear	0.093	0.057	0.125	0.009	0.004
Quadratic	0.699	0.038	0.057	0.079	0.019
Third week	**Egg weight (g)**	**Egg mass (g)**	**Feed intake**	**FCR**	**Egg production (%)**
Basal diet	12.17	582.14	38.05	3.660	81.147
Basal diet + 0.1% mealworm powder	12.08	561.06	36.27	3.627	85.357
Basal diet + 0.2% mealworm powder	12.32	569.25	35.42	3.486	82.500
SEM	0.107	13.989	0.810	0.055	1.706
P-value	0.323	0.576	0.104	0.102	0.458
Linear	0.362	0.527	0.040	0.046	0.259
Quadratic	0.231	0.409	0.650	0.441	0.617
Fourth week	**Egg weight (g)**	**Egg mass (g)**	**Feed intake**	**FCR**	**Egg production (%)**
Basal diet	12.535	614.78	40.41	3.601	84.81
Basal diet + 0.1% mealworm powder	12.792	601.51	38.79	3.522	89.62
Basal diet + 0.2% mealworm powder	12.737	588.77	38.56	3.583	84.73
SEM	0.084	12.198	0.973	0.118	2.117
P-value	0.119	0.694	0.970	0.754	0.273
Linear	0.117	0.402	0.883	0.464	0.123
Quadratic	0.159	0.991	0.848	0.940	0.709
Fifth week	**Egg weight (g)**	**Egg mass (g)**	**Feed intake**	**FCR**	**Egg production (%)**
		**	NS	NS	NS
Basal diet	12.064	521.24^{b}	40.00	4.08	79.90
Basal diet + 0.1% mealworm powder	12.194	546.28^{ab}	37.89	3.78	82.29
Basal diet + 0.2% mealworm powder	12.534	571.51^{a}	40.47	3.87	83.77
SEM	0.094	8.817	0.750	0.143	2.425
P-value	0.011	0.005	0.070	0.340	0.792
Linear	0.004	0.001	0.664	0.308	0.512
Quadratic	0.381	0.992	0.025	0.288	0.936
1-5 week	**Egg weight (g)**	**Egg mass (g)**	**Feed intake**	**FCR**	**Egg production (%)**
					*
Basal diet	12.200	568.87	38.92	3.787	81.523^{b}
Basal diet + 0.1% mealworm powder	12.397	573.02	38.25	3.680	84.929^{a}
Basal diet + 0.2% mealworm powder	12.530	556.57	38.01	3.832	80.202^{b}
SEM	0.107	1.1341	0.411	0.0472	1.105
P-value	0.132	0.628	0.627	0.121	0.037
Linear	0.049	0.493	0.622	0.286	0.014
Quadratic	0.809	0.507	0.434	0.075	0.565

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05) and ** (P<0.01).
NS: non significant.
SEM: standard error of the means.

Table 3 Effects of mealworm (*Tenebrio molitor*) on egg quality traits of laying quails (*Coturnix coturnix Japonica*) during the whole experiment

Item	Egg length (mm)	Egg width (mm)	Shell weight (g)	Yolk diameter (mm)	Yolk weight (g)	Albumen weight (g)
Basal diet	33.58	25.83	1.202	24.59	3.865	6.209
Basal diet + 0.1% mealworm powder	33.71	25.84	1.226	24.58	3.851	6.322
Basal diet + 0.2% mealworm powder	33.67	25.80	1.234	24.27	3.749	6.518
SEM	0.725	0.949	1.606	1.610	1.548	1.469
P-vlue	0.927	0.993	0.585	0.813	0.350	0.100
Linear	0.786	0.937	0.460	0.576	0.191	0.037
Quadratic	0.788	0.940	0.581	0.767	0.555	0.722

Item	Albumen height (mm)	Yolk height (mm)	Yolk index	Shape index	Haugh unit
	***	***			
Basal diet	6.243	13.838	0.563	76.93	97.77
Basal diet + 0.1% mealworm powder	6.720	14.645	0.596	76.63	99.78
Basal diet + 0.2% mealworm powder	6.792	14.989	0.618	76.61	100.26
SEM	3.347	2.606	0.0196	0.599	1.002
P-value	0.2021	0.1285	0.180	0.915	0.218
Linear	0.103	0.050	0.071	0.713	0.105
Quadratic	0.467	0.625	0.830	0.852	0.543

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
*** (P<0.001).
NS: non significant.
SEM: standard error of the means.

Table 4 Effects of mealworm (*Tenebrio molitor*) on antibody titer against sheep red blood cell (SRBC) in laying Japanese quails (89 and 96 d of age, $\log_2$)

Item	On 89 d of age	On 96 d of age
Basal diet	2.045[b]	2.995[b]
Basal diet + 0.1% mealworm powder	2.787[a]	3.477[a]
Basal diet + 0.2% mealworm powder	3.026[a]	3.652[a]
SEM	0.112	0.059
P-value	0.0001	< 0.0001
Linear	< 0.0001	< 0.0001
Quadratic	0.093	0.058

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

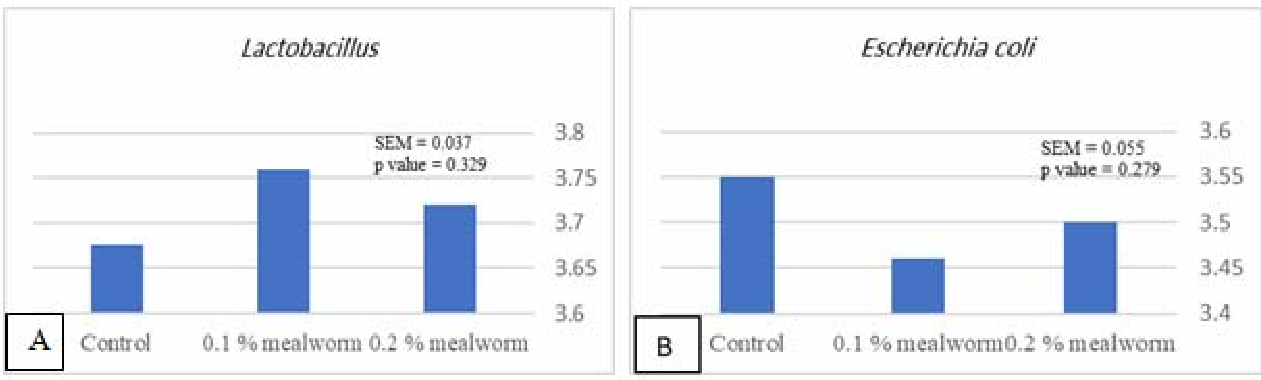

Figure 1 Effects of mealworm (*Tenebrio molitor*) on ileal *Lactobacillus* (A) and *Escherichia coli* (B) population in laying *Japanese* quails at the end of the experiment (96 d of age, $\log_{10}$ CFU g^{-1})

In this study, adding insect powder to laying Japanese quails' diet improved their antibody titer against SRBC as a primary and secondary response compared to control group.

However, there was no difference between antibody titer of the groups fed with 0.1% and 0.2% mealworm. Similar to our findings, Benzertiha *et al.* (2020) reported that addition of 0.2% and 0.3% of *T. Molitor* and *Zophobas morio* full-fat meals changed the immune system traits. Nowadays, it is well known that some insects can synthesize antimicrobial peptides that are helpful substances for innate immune defense. Also, insects have a high content of chitin, which has antibacterial effects which can promote the efficiency of the natural immune system (Esteban *et al.* 2001; Xu *et al.* 2013). Also, Lee *et al.* (2008) found that chitin has different biological activities, such as immune-stimulation. Chitin as a polysaccharide may be a substrate for microbial fermentation in the gastrointestinal tract of the chickens. It could serve as a substrate for the production of chitosan, which can have immunomodulatory, antioxidative, antimicrobial, and hypocholesterolemic effects when used as a feed additive for poultry (Swiatkiewicz *et al.* 2015). Chitin consists mainly of β-(1→4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose units and partially of β-(1→4)-linked 2-amino-2-deoxy-β-D-glucopyranose. It is the second most abundant polymer in the world after cellulose. Chitin is insoluble in water, diluted mineral acids and most organic solvents. It has a low reactivity, and low processability (Veldkamp *et al.* 2012). Tabata *et al.* (2017) reported that chicken has the acidic chitinase degrades chitin in dimers of *N*-acetyl-D-glucosamine and produces chito-oligosaccarides.

Nogales-Mérida *et al.* (2019) reported that insect derivate products including protein concentrates, chitins, oils and antimicrobial peptides not only enhance the growth performance, but may also boost the fish's immunity. Also, it was reported that *Hermetia illucens* has antibacterial activity (Park *et al.* 2014; Park *et al.* 2015), and its low doses may promote the immunity response similar to low doses of dietary antibiotics (Gadde *et al.* 2017). Islam and Yang (2016) noted that the immunoglobulin levels of broiler chicks were increased after the supplementation of 0.4% of *Tenebrio molitor* and *Zophobas morio* probiotics obtained by fermenting the insect meals with *L. plantarum* and *S. cerevisiae*. The albumin/globulin ratio (a sign of immune response) decreased when broilers fed on insect meal diet. This is maybe due to the prebiotic effects of chitin (Bovera *et al.* 2015). Parallel to the findings of present study, Lee *et al.* (2018) found that using black soldier fly larvae in broiler chicks' diet could increase CD4+ lymphocyte, serum lysozyme activity, spleen lymphocyte proliferation, and promote the broilers' immune responses.

Regards to the results of the present study, mealworm supplementation did not have any significant effect on ileal *Lactobacillus* and *Escherichia coli* population in laying Japanese quails after five weeks of the insect consumption. Stoops *et al.* (2016) studied the microbiota of *Tenebrio molitor* and found that total aerobic counts were 8 ± 0.3 log CFU/g, *Enterobacteriaceae* 7.23 ± 0.4, *Lactic bacteria* 7.47 ± 0.15 CFU/g.

Borrelli *et al.* (2017) found that fermentable chitin can be considered as a potential prebiotic that promotes a healthy state and microbiota in laying hens. It is well known that chitin is the second most frequent polysaccharide in the world with β-1, 4-linked *N*-acetyl-D-glucosamine (Borrelli *et al.* 2017).

Chitin is a substrate for some beneficial intestinal microbiota. It was reported that using shrimp meal up to 15%, which contain chitin, increased intestinal *Lactobacillus* and decreased intestinal *Escherichia coli* and cecal *Salmonella* (Khempaka *et al.* 2011). Van Huis (2013) demonstrated that using insects in poultry diet could help to decrease antibiotics usage; thus, insects may help to control antibiotic resistance in poultry and their consumer. Larvae of *Tenebrio molitor* have proteins (tenecins) with antimicrobial activity.

These substances are active against gram-positive bacteria: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus pyrogen*, *Micrococcus luteus*, *Corynebacterium diphtheria* (Jozefiak *et al.* 2016b). In recent research, Józefiak *et al.* (2018) found that the inhibition of possibly pathogenic bacteria and the positive modulation of microbiota in the broiler chicken gastrointestinal tract (GIT) could be due to full-fat insect meals, potentially similar to the effects of ionophore coccidiostats, i.e., Salinomycin.

In the present study, adding mealworm to laying quails' diet decreased the ileal population of *Escherichia coli* numerically, but it was not significant. We conclude that mealworm supplementation up to 0.2% in a five-week period was not sufficient to change ileal microbiota of laying quails under normal rearing conditions. More research is needed to determine the optimum level of mealworm usage in order to modify intestinal microbial count.

CONCLUSION

According to our results, adding mealworm at the level of 0.1% increased egg weight, egg production, albumen weight, and yolk height in Japanese laying quails. Mealworm powder increased antibody titer against SRBC in laying quails on 89 and 96 d of age. It seems that full-fat mealworm powder has the potential to be considered as a novel organic additive in quails´ diet.

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