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Effect of Bee Pollen Supplemented Diet on Performance, Egg Quality Traits and some Serum Parameters of Laying Hens

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ABSTRACT

This study was conducted to evaluate the effect of bee pollen (BP) supplemented diet on performance, egg quality and some blood parameters of laying hens. A total of 96 Lohmann layers, 28 weeks of age, were randomly allocated to four dietary treatments and fed a control diet (Pol-0), basal diet plus 0.5% (Pol-1), 1.0% (Pol-2) and 1.5% (Pol-3) bee pollen. The present study lasted for twelve weeks. During the experimental period, the hens were fed *ad-libitum* and water was available all the times. The hen house was lit for 17 h. The layers fed with diets including BP showed linear increases in final body weight and body weight changes calculated from initial and final body weights at the end of the trial. The supplementation of bee pollen into layer diet linearly decreased feed intake and linearly increased feed conversion ratio. The inclusion bee pollen at different levels affected the egg weight (quadratic), yolk index (linear), Haugh unit and albumen index (cubically). On the other hand, supplemental bee pollen linearly decreased cholesterol, triglyceride and P values and linearly increased Mg value in the serum parameters. However, the other serum parameters were not affected by the treatment. In conclusion, supplementation of bee pollen during the study period improved the feed conversion ratio and decreased serum cholesterol and lipid contents. In addition, according to economic analysis, the net income for hen fed 1.5% BP addition diet increased by about 0.62% compared to control group. Therefore, bee pollen may be used in laying hen diets because of its psitive effects on FCR, serum cholesterol and lipid contents.

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Bee pollen, Egg quality, Laying hens, Performance, Serum parameters

INTRODUCTION

Antibiotics have long been used for poultry production to treat and to prevent disease, to improve feed efficiency in conventional livestock (Allen *et al*., 2013). But their use in feed diets of productive livestocks leads to the formation of resistance against bacteria, and pathogenic for humans. So that the researchers developed and used some additives to improve performance parameters. Non-antibiotic alternatives to antibiotic growth promoters have been proposed for use in animal diets due to their safety in both animals and humans (Kaya *et al*., 2014).

Alternatives to antibiotics are urgently needed in animal nutrition (Allen *et al*., 2013; Chen *et al*., 2020). In recent years, the use of natural products as substitutes for replacement of antibiotics for improving the performance and immune system in animal life is being encouraged. One of the regarded candidates in natural products is bee pollen (Farag and El-Rayes, 2016).

BP is a collection of flower pollen from various botanical sources, which are collected and mixed with nectar and honeybee salivary substances, carried out by worker bees and left at the hive's entrance (Almedia *et al*., 2017). BP contains some importent nutrients and bioactive compounds: proteins, which are among the main components of bee pollen, include enzymes and both essential and nonessential amino acids, lipids, carbohydrates, phenolic compounds, minerals, some antioxidant vitamins, such as C, E, β-carotene, as well as vitamins from the B-complex (Sattler *et al*., 2015; Conte *et al*., 2017; Ares *et al*., 2018; Rizk *et al*., 2018). In addition, Al-Osaimi *et al*. (2018) reported that BP is a rich source of potassium (K^+), phosphorus (P), magnesium (Mg^{2+}), calcium (Ca^{2+}), sodium (Na^+), sulfur (S), iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), chromium (Cr) and nickel (Ni). BP mixture is formed by 13-55% carbohydrates, 10-40% protein, 1-20% lipids, 3-8% water, 0.5-3% minerals, 0.02-0.1% vitamins, 0.04-3% flavanoids and other compounds such as resins and antibiotic substances (Farag and El-Rayes, 2016). Also, Attia *et al*. (2011) stated that bee pollens are rich sources of essential amino acids; oil (6%), containing more than 51% polyunsaturated fatty acids (PUFA) consisting of 39% linolenic, 20% palmitic and 13% linoleic acids,

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more than 12 vitamins, 28 minerals, 11 enzymes or co-enzymes, 11 carbohydrates (35-61%) which are mainly glucose, fructose and sucrose, flavonoids and carotenoids, phytosterols. In reality the chemical compositions of BP correlates with the plant species from which the pollen was collected (Taha, 2015), the environmental conditions, age and nutritional status of the plants during the flowering period (Farag and El-Rayes, 2016). BP promotes animal growth, improves the quality of animal products and security, enhances immunizing function of poultry and protects intestinal tract health (Farag and El-Rayes, 2016).

The present study was carried out in order to determine whether inclusion of bee pollen supplemented diets during the peak period improves performance, egg quality and serum parameters of laying hens.

MATERIALS AND METHODS

Animals, diet and management

Lohmann white layers (n=96), 28 weeks of age, were randomly divided into 4 groups, with 6 replicate cages (50 × 46 × 46 cm) as subgroups, each comprising four hens, and fed a basal diet (Pol-0), basal diet plus 0.5% (Pol-1), 1.0% (Pol-2) and 1.5% (Pol-3) BP for 12 weeks. The BP was obtained from the Beekeeping Branch of the Agricultural Faculty, Food and Livestock Application Research Center of Atatürk University. Bee hives were kept and managed on the pasture of Yeşildere village of Erzurum Yakutiye District at the spring period. The basal diet was formulated to meet nutrient requirements of laying hens according to NRC (1994) recommendations. The experimental diets were analyzed using AOAC (1990) methods. Ingredients and chemical compositions of basal diet and BP are given in Table I, hens were fed *ad libitum* once daily at 08:30 h and water was available all the times during the experimental period (12 weeks). Layers were exposed to a 17-h daily photoperiod.

Sample collection and analytical procedure

Body weights were recorded at the beginning and at the end of the study. Feed intake and egg production were recorded daily, egg weight was measured bi-weekly. Feed conversion ratio (FCR) was calculated as kilogram of feed consumed per kilogram of eggs produced. Egg samples were collected regularly once a month (n=12 per group) and stored for 24 h at room temperature to determine egg quality parameters such as shape index, shell strength, shell thickness, albumen index, yolk index, yolk color (Yolk Colour Fan, the CIE standard colorimetric system, F. Hoffman-La Roche Ltd., Basel, Switzerland), and Haugh unit. Egg quality traits were assessed according to the method of Kaya and Macit (2012).

At the end of the experiment, blood samples (5.0 mL) were collected from the axillary vein from 6 hens from each group (twenty-four hens) into non-heparinized tubes using sterilized needles. Blood samples were incubated at 37°C for 2 h and then centrifuged at 3,000 r for 5 min. The serum samples were stored in 1.5 mL Eppendorf tubes for the determination of serum parameters. Serum parameters including cholesterol, triglycerides, glucose, total protein, albumin, AST, ALP, ALT, Ca, P and Mg, were measured using commercial kits (DDS® Spectrophotometric Kits, Diasis Diagnostic Systems Co., İstanbul, Turkey) with autoanalyzer Mindray perfect Plus 400.

Economical efficiency

The economical efficacy was calculated by using the data related to performance parameters of hens fed on diets with 0.5, 1.0 and 1.5% bee pollen and without bee pollen during the experimental period and give in Table V.

Statistical analysis

Data were subjected to ANOVA using GLM procedure of SPSS 10.0 software (2011). Polynomial contrast was constructed to determine the effect of BP in the level diets. The effects of the dietary treatments on response variables were declared to be significant at $P < 0.05$. The simple GLM used was as follows:

$$Yij = \mu + ai + eij$$

where μ is overall mean, ai effect of the diet, i and eij the residual error.

RESULTS AND DISCUSSION

Laying performance

The performance parameters of laying hens fed on diets supplemented with 0%, 0.5%, 1.0% and 1.5% in response to the dietary supplementation with 0.5%, 1.0%, 1.5% levels of BP are given in Table II. BP supplemented diets linearly decreased feed consumption compared to the control group ($P < 0.05$).

This result is in agreement with the finding of El- Naga *et al*. (2014) who found that hens fed with diets including 1 or 2% bee pollen consumed less feed compared to control group fed on basal diet. Results from the present study were similar to the finding of Rizk *et al*. (2018) who studied the diets supplemented with 500, 1000 and 1500 mg/kg diet of BP on performance of laying hens and reported that the hens fed with diet including 1500 mg BP/kg diet consumed less feed than that of the control group. Unlike these findings, Hosseini *et al*. (2016) stated that addition of 20 g/kg diet of BP into broiler starter diet increased average daily feed intake of broilers. Also, Canoğulları *et al*. (2009) reported that the diets supplemented with

Table I. Ingredients and chemical compositions of the experimental diet and bee pollen (%).

Ingredient		(%)	Analyzed Chemical composition (on a DM basis)		
Corn		59.63	Dry matter (%)		88.36
Soybean meal (46% CP)		19.50	Crude protein (%)		17.58
Sunflower seed meal (%36 CP)		7.40	Crude fiber (%)		3.19
Soybean oil		1.49	Crude ash (%)		13.77
Meat-bone meal		1.50	Fat (%)		3.75
Monocalcium phosphate		0.07	Calcium (%)		3-4
Marble		9.50	Phosphorus (%)		0.70
Vitamin-mineral premix[1]		0.30	Metabolizable energy (kcal/kg)[4]		2724
Salt (NaCl)		0.20			
Sodium bicarbonate		0.15			
Ekobond (Toxin binder)[2]		0.10			
Salmonil LCT (Organic Acid Mixture)[3]		0.10			
DL-Methionine		0.06			
Chemical composition of bee pollen (%)					
Dry matter	Ether extract		Crude Ppotein	Crude ash	
86.06	9.47		24.07	3.02	

[1]4.000.000 IU Vitamin A; 800.000 IU cholecalciferol (Vit D3),10.000 mg α-tocopheryl acetate (Vit E); 1.333 mg menadione sodium (Vit K3); 1.000 mg thiamine mononitrate (Vit B1); 1.667 mg riboflavin (Vit B2); 8.333 mg niacin (Vit B3); 3.333 mg Ca-D- panthotenic acid (Vit B5); 1.667 mg pyridoxine (Vit B6); 333 mg folic acid (Vit B9); 5 mg cyanocobalamin (Vit B12); 15 mg D-biotin (Vit H); 16.667 mg Ascorbic acid (Vit C);100.000 mg Choline chloride; 200 mg Lutein; 12.5 mg Zeaxanthin; 26.667 mg manganese oxide; 20.000 mg Zinc oxide; 20.000 mg İron sulfate; 1.667 mg Copper sulfate; 67 mg Cobalt carbonate; 333 mg calcium iodate; 50 mg Sodium selenite; 300 mg hydroxy methionine.
[2]Contained sepiolite, 1000.000 mg/kg (mycobond, optimite, Nottinghanshire, UK.).
[3]Contained formic acid 35 000 mg/kg; ammonium propionate, 85 000 mg/kg; ammonium formate 160 000 mg/kg; propionic acid, 20 000 mg/kg; sodium aluminosilicate as carrier, 700 000 mg/kg (Salgard PowderTM, Optivite, Nottinghamshire, UK).
[4]It was calculated according to TSE (1991).

Table II. Performance parameters of laying hens fed diets including bee pollen at different levels.

Treatment	Feed consumption (g/d)	Egg production (%)	Egg weight (g)	FCR (kg feed/kg egg)	Body weigth (g)		
					Initial	Final	Change
Pol-0	124.00	89.53	65.60	2.11	1614.8	1619.2	4.4
Pol-1	123.39	89.30	66.72	2.07	1616.3	1665.7	49.4
Pol-2	122.01	90.55	67.08	2.03	1608.3	1710.0	101.7
Pol-3	118.82	90.29	65.33	2.01	1611.5	1723.3	111.8
SEM	1.39	2.26	0.78	0.03	23.72	26.63	29.18
Polinomial analyses							
L	0.014	0.730	0.895	0.034	0.868	0.007	0.010
Q	0.368	0.994	0.080	0.793	0.972	0.540	0.559
C	0.871	0.770	0.701	0.860	0.849	0.811	0.707

Pol-0, Control; Pol-1, 0.5% Bee pollen; Pol-2, 1.0% Bee pollen; Pol-3, 1.5% Bee pollen; L, Linear; Q, Quadratic; C, Cubic; FCR, Feed conversion ratio (kg feed consumed per kg egg produced); SEM, Standart error of mean.

5, 10 and 20 g/kg diet of BP significantly increased feed consumption by quail chicks.

The inclusion of BP had no effect on egg production and egg weight parameters ($P > 0.05$). In accordance with results of present study, Arpasova *et al*. (2013) found that diet including 0.4 g/kg pollen extract did not affect the egg production and egg weight of laying hens. In contrast of this sutdy, Wang *et al.* (2007) noted that egg weight significantly increased in laying hens fed on diet supplemented with 1.5% BP (El-Naga, 2014). Feed conversion ratio linearly decreased with increasing level of BP supplementation (P<0.05). Similarly, supplemental BP improved FCR when laying hens fed with diets containing up to 1-2 % bee pollen (El- Naga *et al.* (2014). The findings of some studies related to FCR in poultry were in agreement with the findings of the present study (Shanoon *et al.*, 2015; Babaei *et al.*, 2016; Rizk *et al.*, 2018). Rizk *et al.* (2018) stated that the feed conversion ratio was significantly decreased by BP supplementation (1500mg/kg) and also reported that this improvement may be attributed to the reduction in feed consumption. Similar to the results of present study, it was reported that quail diets supplemented with 5, 10 and 20 g/kg diet BP decreased feed efficiency with increased additional BP, although the differences in FCR among the groups were not significant (Canoğulları *et al.*, 2009).

In response to increased supplemental BP, final body weight and body weight change linearly increased (P<0.01). This result may be attributed to several enzymes inside of BP which support the digestive system to improve the FCR. In the previous studies being similar to the current study, it was pointed out that live weight gains of broiler and Japanese quail increased with bee pollen supplemented rations (Canoğulları *et al.*, 2009; Seven *et al.*, 2011; Babaei *et al.*, 2016; Hosseini *et al.*, 2016). Also, Hascik *et al.* (2012) noted that broilers fed with diets including 400 mg/kg and 800 mg/kg BP had higher body weight than that of the control group fed on basal diet.

Egg quality parameters

The effects of supplemental BP (0, 0.5, 1.0, 1.5%) on egg quality traits of laying hens are presented in Table III. As supplemental BP in diet increased, egg weight from egg quality parameters quadratically increased. As seen in Table III, shape index, shell strength, shell thickness, shell weight and yolk color parameters were not affected by the dietary treatments. Yolk index linearly increased with increasing level of supplemental BP (P<0.05). Increased BP in the diet had a cubic effect on albumin index (P<0.01) and Haugh unit (P<0.05). The Haugh unit, an indicator of the most widely accepted measure of internal quality, tended to be decreased according to the elapsed time of storage. General nutrients in layer feed did not appear to have any beneficial effect on Haugh unit (Naber, 1979), but it was suggested that certain natural antioxidants such as vitamin C, vitamin E and selenium may be beneficial to albumen quality by their antioxidant properties (Lim *et al.*, 2006). According to the available literature, BP contains precourse of vitamins B complex, C, D, E vitamins and carotenoids such as carotene, which can be pro-vitamin A (Oliveira *et al.*, 2009; Melo *et al.*, 2010; Rizk *et al.*, 2018). Phenolic compounds have recently received much attention for their wide range of functions such as antimicrobial, antidiabetic, antihyperlipidemia or antiinflammatory effects; yet the main activity reported for these compounds has been as antioxidants. BP possesses a wide range of phenolic compounds such as rutin, quercetin, vanillic acid, and protocatechuic acid; nevertheless, its composition varies because of its botanical and geographic origins as well as other factors such as soil type, weather conditions, and beekeeper activities. Several phenolic compounds like rutin, quercetin, caffeic acid, resveratrol or kaempferol were detected in bee pollen samples from different countries (Ares *et al.*, 2018). BP is a rich source of flavonoids, carotenoids and phytosterols (Hosseini *et al.*, 2016). In this study, the positive effects of BP, as a natural antioxidant, on albumen index and Haugh unit can be attributed to the antioxidant properties, which have several bioactive secondary metabolites.

Similar to the results of this study, it was reported that BP supplemented (1-2%) layer diet did not affect shape index, shell thickness, yolk color parameters, but Haugh unit significantly increased in pollen groups compared to control (El-Naga, 2014). Our results from present study are in agreement with the findings of Arpasova *et al.* (2012) who stated that while Haugh unit was significantly higher, most of the egg internal quality parameters were not significantly affected by supplemental bee pollen. In another experiment, Arpasova *et al.* (2013) studied the effect of diet including bee pollen 0.4 g/kg diet on egg quality parameters of laying hens and pointed out that eggs weight, yolk color and yolk index were not influenced by supplamental BP. A study related to the effect of BP supplementation (500, 1000 and 1500 mg/kg diet) on egg quality traits of Sinai hens showed that the bee pollen supplemented diets did not have significant effect on egg shape index, shell weight, shell thickness, albumen index and yolk index.

Some serum parameters

The effects of the experimental diets supplemented with BP on serum parameters are summarized in Table IV. As showen in Table IV, glucose, total protein, albumin, aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), calcium (Ca) did not differ among the experimental groups. Serum cholesterol (P<0.01), triglycerides (P<0.01) concentration decreased and Mg (P<0.05) concentration increased linearly with increasing level of BP supplementation. These findings were agree with those of Rizk *et al.* (2018) who

Table III. Egg quality parameters of laying hens fed diets including BP at different levels.

Treatment	Egg weight (g)	Shape index (%)	Shell strength (kg/cm²)	Shell thickness (cm×10⁻²)	Shell weight (g)	Yolk color	Yolk index (%)	Albumen index (%)	Haugh unit
Pol-0	63.87	73.92	2.96	0.40	8.14	12.11	41.22	10.04	85.55
Pol-1	69.57	72.86	3.11	0.39	8.48	12.00	42.55	8.49	80.13
Pol-2	69.07	74.28	3.14	0.39	8.55	11.83	42.94	9.92	86.49
Pol-3	67.45	74.94	3.17	0.39	8.70	11.77	43.09	9.23	83.98
SEM	1.42	0.61	0.25	0.02	2.44	0.16	0.55	0.443	1.84
Polinomial analyses									
L	0.122	0.113	0.563	0.295	0.123	0.122	0.044	0.620	0.844
Q	0.018	0.172	0.820	0.863	0.692	0.865	0.268	0.346	0.440
C	0.433	0.249	0.905	0.851	0.725	0.820	0.977	0.019	0.021

For abbreviations, see Table II.

Table IV. Serum parameters of laying hens fed diets including BP at different levels.

Treatment	Chol [2]	Trg [2]	Glu [2]	TP [1]	Alb [1]	AST [3]	ALP [3]	ALT [3]	Ca [2]	P [2]	Mg [2]
Pol-0	145.6	1329.2	221.0	5.7	1.58	192.6	1020.2	1.6	26.42	4.5	3.34
Pol-1	124.6	1240.6	220.2	5.28	1.62	186.0	647.6	1.8	24.96	4.1	3.46
Pol-2	117.2	1163.2	230.0	5.30	1.54	201.4	1070.4	1.6	26.22	3.9	3.58
Pol-3	99.8	972.0	227.8	5.48	1.50	181.2	1399.4	1.4	23.38	3.6	3.76
SEM	4.80	24.73	7.54	0.30	0.05	11.78	324.54	0.48	1.33	0.33	0.12
Polinomial analyses											
L	0.00	0.00	0.38	0.64	0.12	0.73	0.30	0.71	0.20	0.05	0.04
Q	0.71	0.77	0.93	0.33	0.40	0.57	0.30	0.68	0.61	0.95	0.75
C	0.29	0.89	0.51	0.84	0.45	0.29	0.55	0.85	0.27	0.85	0.25

Chol, cholesterol; Trg, triglycerides; Glu, glucose; TP, total protein; Alb, albumin; AST, aspartate aminotransferase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; Ca, Calcium; P, Phosphorus and Mg, Magnesium; [1]g/dL; [2]mg/dL; [3]unit/L. For abbreviations, see Table II.

Table V. Economical comparison among the groups fed with diets in absence or presence of bee pollen at different levels.

Groups		Pol-0	Pol-1	Pol-2	Pol-3
TEN(28-40wks)		75.21	75.01	76.06	75.84
TFC (28-40wks)		10.416	10.365	10.249	9.981
Cost/TL	Feed	10.416 kg×2.2TL = 22.92TL	10.365 kg×2.2TL = 23.22TL	10.249 kg×2.2TL = 22.55TL	9.981 kg×2.2TL = 21.96TL
	BP price/TL	0	5.18 g × 0.08TL = 0.42TL	10.249 g × 0.08TL = 0.82TL	14.972 g×0.08TL = 1.20TL
	Total	22.92 TL	23.22 TL	23.37 TL	23.16 TL
Total revenue /hen		75.21egg×0.60TL =45.13 TL	75.01egg×0.60 TL =45.01TL	76.06egg×0.60TL =45.64 TL	75.84egg×0.60 TL =45.50 TL
Net income (TL)		22.21TL	21.79TL	22.27TL	22.35TL
RD %		100	98.10 (-1.90)	100.26 (+0.26)	100.62 (+0.62)

Total revenue/hen, Egg number×egg price; Net income (TL), Total revenue-Total cost; RD%, Relative deference; TL, Turkish Lira; One kg of bee pollen, 80 TL; Egg price at marketing, 0.60 TL; Total revenue/hen, (egg number×egg price); Net income (TL), Total revenue-Total cost; RD%, Relative deference. For abbreviations, see Table II.

indicated that the Sinai laying hen diets supplemented with BP at different levels (500, 1000, 1500 mg/kg) significantly decreased the total cholesterol and triglycerides compared to control group, but no effects of experimental diets on serum total protein, albumin and globulin. The decreased serum cholesterol and triglycerides values may be due to unsaturated fatty acids such as oleic, linoleic and linolenic inside of bee pollen that inhibit the accumulation of lipid peroxidation product (Rizk *et al*., 2018). In response to increasing level of BP supplementation, serum Mg concentration linearly increased ($P < 0.01$) in this study.

In another study, El-Naga (2014) showed that increased levels of bee pollen (1% and 2%) in diet decreased the glucose from serum parameters, but there were not significant differences among the treatment groups in terms of serum protein, albumen, globulin and total lipids of Norfa laying hens.

Similar to the present study, Attia *et al*. (2011) stated that the addition of bee pollen (100, 200, 300 mg/kg body weight) to white rabbit rations reduced blood triglycerides and cholesterol levels.

Regarding to economical efficacy, the findings in Table V showed that laying hens fed with diet containing BP at 1.5% level during the experimental period had higher net income than those of control and other treatment groups.

CONCLUSION

In this study, supplemental bee pollen being natural resource had significant effects on some performance and serum parameters including improved FCR and Haugh unit, and reduced serum cholesterol and triglycerides in laying hens. In addition, according to economic analysis results, the net income calculated for hens fed with 1.5% BP addition diet increased by about 0.62% compared with control group. Teherefore, bee pollen may be used in diets of laying hens to improve some performance and serum lipid parameters and to increase net income of layer flock.

Statement of conflict of interset

The author declears there is no conflict of interset.

REFERENCES

Allen, H.K., Levine, U.Y., Looft, T., Bandrick, M. and Casey, T.A., 2013. Treatment, promotion, commotion: antibiotic alternatives in food-producing animals. *Trends Microbiol.*, **21**: 114-119. https://doi.org/10.1016/j.tim.2012.11.001

Almeida, J.F., Reis, A.S., Heldt, L.F.S., Pereira, D., Bianchin, M., Moura, C., Plata-Oviedo, M.V., Haminiuk, C.W.I., Ribeiro, I.S. Luz, C.F.P. and Carpes, S.T., 2017. Lyophilized bee pollen extract: A natural antioxidant source to prevent lipid oxidation in refrigerated sausages. *LWT – Fd. Sci. Technol.*, **76**: 299-305. https://doi.org/10.1016/j.lwt.2016.06.017

Al-Osaimi, M., El-Ansary, A., Al-Daihan, S., Bhat, R.S. and Bacha, A.B., 2018. Therapeutic and protective potency of bee pollen against neurotoxic effects induced by prenatal exposure of rats to methyl mercury. *J. mol. Neurosci.*, **65**: 327–335. https://doi.org/10.1007/s12031-018-1107-1

AOAC, 1990. *Official methods of analysis*. Vol.1. 15th ed. Arlington, VA.

Ares, A.M., Valverde, S. and Bernal, J.L., 2018. Extraction and determination of bioactive compounds from bee pollen. *J. Pharm. Biomed. Anal.*, **147**: 110–124. https://doi.org/10.1016/j.jpba.2017.08.009

Arpasova, H., Kacaniova, M., Hascik, P. and Sidlova, V., 2012. Effect of selected feed additives on internal quality parameters of table eggs. *Potravinarstro,* **6**: 50-52. https://doi.org/10.5219/235

Arpasova, H., Kacaniova, M. and Galik, B., 2013. The effect of oregano essential oil and pollen on egg production and egg yolk qualitative parameters. *Anim. Sci. Biotecch.*, **46**: 12-16.

Attia, Y.A., Al-Hanoun, A. and Bovera, F., 2011. Effect of different levels of bee pollen on performance and blood profile of New Zealand white bucks and growth performance of their offspring during summer and winter months. *J. Anim. Physiol. Anim. Nutr.*, **95**: 17–26. https://doi.org/10.1111/j.1439-0396.2009.00967.x

Babaei, S., Rahimi, S., Torshizi, M.A.K., Tahmasebi, G. and Miran, S.N.K., 2016. Effects of propolis, royal jelly, honey and bee pollen on growth performance and immune system of Japanese quails. *Vet. Res. For.*, **7**: 13 – 20.

Canogullari, S., Baylan, M., Sahinler, N. and Sahin, A., 2009. Effects of propolis and polen supplementations on growth performance and body components of Japanese quails (*Coturnix coturnix japonica*). *Arch. Geflugelk.*, **73**: 173-178.

Chen, M., Mahfuz, S., Cui, Y., Jia, L., Liu, Z. and Song, H., 2020. The antioxidant status of serum and egg yolk in layer fed with mushroom stembase (*Flammulina velutipes*). *Pakistan J. Zool.,* **52**: 389-392. https://dx.doi.org/10.17582/journal.pjz/2020.52.1.sc6

Conte, G., Benelli, G., Serra, A., Signorini, F., Bientinesi, M., Nicolella, C., Mele, M. and Canale, A., 2017.

Lipid characterization of chestnut and willow honeybee-collected pollen: impact of freeze-drying and microwave-assisted drying. *J. Fd. Comp. Anal.,* **55**: 12-19. https://doi.org/10.1016/j.jfca.2016.11.001

El-Naga, M.K.A., 2014. Effect of bee pollen supplementatıon on productıve and reproductıve performance of norfa chıcken. *Egypt. Poult. Sci*., **34**: 119-132.

Farag, S.A. and El-Rayes, T.K., 2016. Effect of bee-pollen supplementation on performance, carcass traits and blood parameters of broiler chickens. *Asian J. Anim. Vet. Adv.,* **11**: 168-177. https://doi.org/10.3923/ajava.2016.168.177

Hascik, P., Elimam, I.O.E., Garlik, J., 2012.The effect of addition bee pollen to feed mixtures on internal fat of broiler Ross 308. *J. Microb. Biotech. Fd. Sci.,* **2**: 246-252.

Hosseini, S.M., Vakili Azghandi, M., Ahani, S. and Nourmohammadi, R., 2016. Effect of bee pollen and propolis (bee glue) on growth performance and biomarkers of heat stress in broiler chickens reared under high ambient temperature. *J. Anim. Feed Sci.,* **25**: 45-51.

Kaya, H., Kaya, A. Gül, M., Ş. Çelebi, Timurkaan, S. and Apaydın, B., 2014. Effects of supplementation of different levels of organic acids mixture to the diet on performance, egg quality parameters, serum traits and histological criteria of laying hens. *Eur. Poult. Sci.,* **78**: 1-12.

Kaya, H. and Macit, M., 2012. Effect of ınclusion of garlic (*Allium sativum*) powder at different levels and copper into diets of hens on performance, egg quality traits and yolk cholesterol content. *Int. J. Poult. Sci*., **11**: 114-119. https://doi.org/10.3923/ijps.2012.114.119

Lim, K.S., You, S.J., An, B.K. and Kang, C.W., 2006. Effects of dietary garlic powder and copper on cholesterol content and quality characteristics of chicken eggs. *Asian-Aust. J. Anim. Sci*., **19**: 582-586. https://doi.org/10.5713/ajas.2006.582

Melo, I.L., Pereira and Almeida-Muradian, L.B., 2010. Stability of antioxidant vitamins in bee pollen samples, *Quim. Nova*, **33**: 514–518. https://doi.org/10.1590/S0100-40422010000300004

Naber, E. C., 1979. The effect of nutrition on the composition of eggs. *Poult. Sci.,* **58**: 518-528. https://doi.org/10.3382/ps.0580518

Oliveira, K.C.L.S., Moriya, M., Azedo, R.A.B., Almeida-Muradian, L.B., Teixeira, E.W., Alves, M.L.T.M.F. and Moreti, A.C.C.C., 2009. Relationship between botanical origin and antioxidants vitamins of bee-collected pollen. *Quim. Nova,* **32**: 1099–1102. https://doi.org/10.1590/S0100-40422009000500003

Rizk, Y.S., Abuoghaba, A.A., Ismail, I.I. and Awadien, N.B., 2018. Egg productıon, egg qualıty traıts and some hematologıcal parameters of sınaı chıcken straın treated wıth dıfferent levels of bee pollen. Egypt. *Poult. Sci*., **38**: 427-438.

Sattler, J.A.G., Melo, I.L.P., Granato, D., Araújo, E., Freitas, A.S., Barth, O.M., Sattler and Almeida-Muradian, A.L.B., 2015. Impact of origin on bioactive compounds and nutritional composition of bee pollen from southern Brazil: a screening study. *Fd. Res. Int.,* **77**: 82-91. https://doi.org/10.1016/j.foodres.2015.09.013

Seven, I., Tatlı-Seven, P., Sur Arslan, A. and Yıldız, N., 2011. The effects of dietary bee pollen on performance and some blood parameters in Japanese quails (*Coturnix coturnix japonica*) breeding under different stocking densities. *J. Fac. Vet. Med. Univ. Erciyes,* **8**:173–180

Shanoon, A.Q., Jassim, M.S., Mohamed, A.H., Latef, M.S. and AL-Raheem, A.M.A., 2015. The effect of using different levels from date palm pollen in diet on productive performance and some eggs quality measurements for layer hens lohman. *Anim. Vet. Sci.,* **3**: 1-4. https://doi.org/10.11648/j.avs.s.2015030601.11

SPSS, 2011. Corp. Released, IBM SPSS Statistics for Windows, Version 20.0, IBM Corp., Armonk, NY.

Taha, E.K.A., 2015. Chemical composition and amounts of mineral elements in honeybee-collected pollen in relation to botanical origin. *J. Apic. Sci*., **59**: 75-81. https://doi.org/10.1515/jas-2015-0008

Wang, J., Zheng, Y., Wu, W., Ban, W., Tang, D. and He-Qian Q., 2007. Effect of bee pollen on performance and thyroid hormone of layers. *J. Anhui Agrotech. Teachers Coll.,* **2**: 1-4.