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ORIGINAL ARTICLE

Impact of supplementing diets with propolis on productive performance, egg quality traits and some haematological variables of laying hens

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Summary

One hundred and twenty eight, 28-weeks-old Lohmann LSL hybrid layers were used in this experiment, which lasted 12 weeks to investigate the effect of propolis supplementation on the productive performance, egg quality traits and haematological variables of laying hens. All hens were randomly classified into four equal experimental groups, eight replicates (4 birds/each). Hens in group 1 were fed on a commercial diet and considered as control group, while those in groups 2, 3 and 4 were fed on the same commercial diet and supplemented with 250, 500 and 1000 mg propolis/kg diet. The obtained results revealed that daily feed consumption/hen increased insignificantly with increasing propolis level than that of the control group. Regarding the means of egg mass and egg production rate, it was observed that the laying hens fed diets containing 250 and 1000 mg propolis/kg significantly ($p < 0.05$) produced more and heaver egg in comparison with control group. External egg quality traits have not affected with increasing the level of propolis, while eggshell weight was significantly ($p < 0.05$) increased. The internal egg quality traits except albumen and yolk percentages increased significantly ($p < 0.05$) with increasing propolis level for treated hens as compared to those in the control. Concerning the haematological parameters, the results showed that the levels of total protein and globulin increased significantly with increasing propolis level, while cholesterol and liver enzymes were significantly decreased ($p < 0.05$). Heterophils count of hens in the treated groups significantly decreased, whereas the lymphocyte count significantly increased, resulting in a decreased H/L ratio than that of the control group. Thus, it could be concluded that the supplementation of 250 mg propolis/kg diet is highly recommended to improving egg production, blood constituent and haematological parameters of the commercial laying hens.

Keywords propolis supplementation, laying hens, egg quality traits, haematological parameters

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Introduction

The use of antibiotics as growth promoters for poultry production has been banned in the European Union, which caused prohibition of using them as protective agents against the emergence of infectious diseases and consequently increasing the economical losses in the poultry industry (Plail, 2006; Perić et al., 2009). Therefore, many researchers tried to find some natural feed additives such as propolis to be used in poultry farms to reduce the expected harmful effects (Kwiecień and Winiarska-Mieczan, 2009; Hegazi et al., 2012).

Propolis (bee glue) is a natural product collected by bees from various plants, particularly from flowers and leaf buds. As known, bees use propolis to overcome the inner walls of the hive and mix it with wax when building combs to protect the colony and larvae from pathogenic micro-organisms (Krell, 1996) as well as the entrance against intruders (El-Bassuony, 2009). Propolis consists of resin and vegetable balsam (50%), wax (30%), essential and aromatic oils (10%) as well as both pollen and other substances (5%) as organic debris (Burdock, 1998). Many researchers explained the effectiveness and the physiological role of propolis against a variety of viruses (Amoros et al., 1994), bacteria (Velikova et al., 2000), fungi (Murad et al., 2002) and moulds (Miyataka et al., 1997).

Also, the findings of Roodsari et al. (2004), Zeng et al. (2004), Guclu-Kocaoglu (2010) and Mathivanan et al. (2013) showed that the use of propolis has a beneficial influence on daily gains, feed intake and conversion in different animal species, including poultry.

A similar trend was also observed by Galal et al. (2008), who found that the averages of egg numbers and egg production rate for hens treated with propolis at 100 and 150 mg/kg diet significantly ($p < 0.05$) increased than those of the control group, while the eggshell thickness for eggs produced from treated laying hens was significantly ($p < 0.05$) higher as compared to the control group. Also, the results of Seven et al. (2011) showed that the dietary supplementations of laying hens with flavomycin or propolis have significantly reduced the negative effects of heat stress on performances, nutrient digestibility and eggshell characteristics ($p < 0.05$).

With regard to the propolis supplementation on plasma cholesterol, the findings of El-Neney et al. (2014) showed that plasma cholesterol was significantly reduced ($p < 0.05$) in Dokki 4 laying hens fed propolis compared to control. Also, they added that plasma total protein, albumin and globulin were significantly ($p < 0.05$) lower for control than those fed propolis.

Referring to blood components, they found that using different dietary propolis levels of treated groups led to a significant increase ($p \leq 0.05$) in RBC and WBC, Hb, lymphocytes, eosinophils and monocytes percentages, while the basophils percentage was insignificantly affected.

In general, the use of propolis is pronouncedly increasing in medical science, but very limited data is available regarding its use in the field of poultry production. Therefore, this experiment aimed to study the effect of supplementing the diets of laying hens with propolis on the productive performance, egg quality traits and haematological parameters.

Materials and methods

This experiment was carried out at the Experimental Poultry Farm, Poultry Production Department, Faculty of Agriculture, Sohag University, Egypt.

Birds, diet and experimental design

One hundred and twenty eight, 28-weeks-old Lohmann LSL hybrid layers were used in this experiment of 84 days. All hens were randomly divided into four equal groups, 8 replicates (4 birds/each). All hens in the group 1 were fed on mash basal diet and considered as the control group (Table 1), while those in the groups 2, 3 and 4 were fed on the same commercial diet, but with the supplementation of 250, 500 and 1000 mg propolis/kg diet, respectively.

Table 1 Composition and calculated chemical analyses of the experimental basal diet

Ingredients	%	Calculated analyses	%
Yellow corn	65.00	Crude protein %	16.00
Soya bean meal (44%)	25.20	ME, Kcal/kg	2748
Limestone	7.50	Crude fibre %	3.32
NaCl	0.34	Crude fat %	2.85
Di-calcium phosphate	1.60	Calcium %	3.28
DL-methionine	0.08	Available phosphorus %	0.35
Premix	0.28	P (available) %	0.38
Total	100	Lysine %	0.92
		Methionine %	0.36
		Met. + Cyct	0.62
		Tryptophan	0.19

Each 3 kg of vitamins and minerals premix contained the following: vitamin A (10 000 000 IU), vitamin E (10 000 mg), vitamin K3 (1000 mg), vitamin D (2 000 000 IU), vitamin B (1000 mg), pantothenic acid (10 000 mg), vitamin B12 (10 mg), vitamin B (1500 mg), vitamin B2 (5000 mg), niacin (30 000 mg), choline chloride (300 000 mg), folic acid (1000 mg), biotin (50 mg), I (300 mg), Mn (60 000 mg), Zn (50 000 mg), Fe (30 000 mg), Cu (4000 mg), Se (100 mg) and Co (100 mg).

All hens were housed in wire galvanized cages (42 W × 50 L × 40 Hcm), equipped with feeders and automatic nipples. They were kept under normal environmental poultry house (22–24 °C and 60–65% RH) and daily exposed to 16 continuous light hours.

The experimental diets were formulated on the basis of a basal diet presented in Table 1.

Measurements and observations

Productive performance

The initial and final body weights (g) were recorded at 28 and 40 weeks of age at the start and at the end of the experiment. Also, number and weight of eggs were daily recorded from 28 to 40 weeks of age. External and internal egg quality traits were measured at 40 weeks of age. Egg weight and eggshell weight were determined using electric balance. Averages of feed consumption (g) were weekly measured. Egg numbers and weights were daily recorded and weighed, while egg mass was calculated by multiplying the numbers of laid eggs and weight (g) for all replicates within each treatment.

Egg quality traits

One hundred and sixty normal eggs were randomly and equally selected on equal basis from eggs laid in the last 2 days of the experiment (4 groups × 8 repli-

cates × 5 eggs/replicate) to assess the egg quality traits. Egg weight, specific gravity, egg surface area, shape index, shell weight, shell percentage and yolk index were tested and determined to measuring the external egg quality traits. Both egg length (long axis) and width (short axis) were measured with the electronic caliper. Yolk index (%), shape index (%), shell percentage (%) and unit surface shell weight (mg/cm²) were estimated by the following equations according to Carter (1975) and Kul and Seker (2004):

$$\text{Yolk index}\,(\%) = (\text{Yolk height}/\text{Yolk diameter}) \times 100,$$

$$\text{Shape index}\,(\%) = [\text{Egg width}\,(\text{cm})/\text{Egg height}\,(\text{cm})] \times 100,$$

$$\text{Shell percentage}\,(\%) = [\text{Shell weight}\,(\text{g})/\text{Egg weight}\,(\text{g})] \times 100,$$

$$\text{Unit surface shell weight}\,(\text{mg/cm}^2) = \text{Egg weight}\,(\text{mg})/\text{Egg surface area}\,(\text{cm}^2).$$

The height of thick albumen (H) and the egg weight (W) were estimated to calculate Haugh units using the formula: Haugh unit (HU) = 100 log (H + 7.57 − $1.7W^{0.37}$), where H = thick albumen height and W = egg weight.

The albumen weight (g) was calculated from the difference between the entire egg weight (g) and the yolk and eggshell weight (g). Yolk diameter along the chalazae line was determined with the caliper (mm). After removal egg contents, the eggshell was dried and weighed to the nearest 0.01 g.

Blood constituents

Blood samples were randomly taken from the brachial vein into heparinized tubes at 11 AM from eight birds per each group at the end of experiment (40 weeks of age). Blood plasma was obtained from the samples by centrifugation for 15 min at 4000 *g* and stored at −20 °C until analysis. The frozen samples was allowed to thaw at room temperature prior to analysis. Total protein, albumin, globulin, triglyceride, cholesterol and liver enzymes were measured by using a biochemical analyzer kits (Technic on RA-XT, New York, USA), while the levels of calcium, sodium and potassium were measured by using flame photometer, model 410, UK.

Haematological parameters

At 40 weeks of age, blood samples were taken from each group for determination of granular and non-granular WBCs and RBCs based on the procedures of Gross and Siegel (1983). Briefly, one drop of blood was being smeared on each of glass slides.

The smears were stained using Wright's stain. One hundred leucocytes, including granular and non-granular, were counted on different microscopic fields representing 100 cells, and the heterophil to lymphocyte ratio was calculated.

Statistical analysis

Data were subjected to a one-way analysis of variance with treatment group effect using the GLM procedure of SAS-6.03 (1998). Significant differences between treatment means were determined using Duncan's new multiple-ranges test (Duncan, 1955).

Results and discussion

Productive performance

The means of body weight, egg weight, egg mass, egg production rate as well as feed consumption and feed conversion ratio of laying hens fed diets supplemented with different levels of propolis are presented in Table 2.

The results showed that the means of final body weight and egg weight of hens that received different levels of propolis were not significantly different in comparison with control group.

In the present study, the findings showed that the propolis supplementation in the diet for laying hens had no significant effect on feed consumption.

This result does not agree with the finding of Guclu-Kocaoglu (2010), who found the laying hens fed diets supplemented with 0.5, 1, 3 and 6 g propolis/kg diet increased significantly the feed consumption than those of the control. Similar results were found by Galal et al. (2008), who found that the feed consumption for laying hens fed diets that contained 100 and 150 mg propolis/kg diet increased significantly compared with control group.

Regarding the egg production rate, the result showed that the propolis treatments of 250, 500 and 1000 mg propolis/kg diet increased significantly ($p < 0.05$) compared to the control group. The highest egg production rate for laying hens in the treated groups could be attributed to the propolis, which contains digestive enzymes (glucose oxidase, catalase and peroxidase), in addition to the pronounced contents of the essential and aromatic oils that may be associated with the improved digestibility of the different nutrients (Khojasteh and Shivazad, 2006).

The current results are in agreement with the findings of Galal et al. (2008), who found that the egg production rate of laying hens, which fed diets supple-

Table 2 Effect of supplementing the diets of laying hens with propolis on the productive performance

	Propolis supplementation (mg/kg diet)					
Traits↓	Control	250	500	1000	p-Value	Sig.
Body weight (g)						
Initial	1617.50 ± 22.6^{a}	1623.91 ± 23.1^{a}	1615.47 ± 21.9^{a}	1621.72 ± 20.5^{a}	0.9928	NS
Final	1643.71 ± 25.0^{a}	1674.22 ± 27.1^{a}	1669.38 ± 22.4^{a}	1680.63 ± 24.6^{a}	0.2215	NS
Egg weight (g)						
28–32 weeks	59.51 ± 0.52^{a}	60.51 ± 0.50^{a}	59.88 ± 0.28^{a}	60.51 ± 0.26^{a}	0.2497	NS
32–36 weeks	60.80 ± 0.45^{a}	61.98 ± 0.59^{a}	61.81 ± 0.35^{a}	62.02 ± 0.27^{a}	0.1703	NS
36–40 weeks	61.01 ± 0.86^{a}	61.99 ± 0.53^{a}	61.93 ± 0.60^{a}	62.76 ± 0.34^{a}	0.2402	NS
28–40 weeks	60.44 ± 0.53^{a}	61.49 ± 0.50^{a}	61.21 ± 0.36^{a}	61.77 ± 0.25^{a}	0.1654	NS
Egg mass (g)						
28–32 weeks	1443.37 ± 40.5^{c}	1597.65 ± 17.8^{ab}	1535.94 ± 25.8^{b}	1637.83 ± 18.3^{a}	0.0001	***
32–36 weeks	1473.99 ± 43.9^{b}	1613.42 ± 41.7^{a}	1583.48 ± 35.2^{a}	1653.37 ± 20.6^{a}	0.0114	*
36–40 weeks	1384.39 ± 50.2^{b}	1559.37 ± 53.1^{a}	1526.57 ± 39.3^{a}	1582.94 ± 34.1^{a}	0.0184	*
28–40 weeks	4302.53 ± 108.4^{b}	4771.46 ± 98.5^{a}	4646.83 ± 71.6^{a}	4875.85 ± 53.2^{a}	0.0004	***
Egg production (%)						
28–32 weeks	86.72 ± 2.53^{c}	94.31 ± 0.81^{ab}	91.63 ± 1.64^{b}	96.65 ± 0.89^{a}	0.0001	***
32–36 weeks	86.61 ± 2.61^{b}	92.97 ± 2.22^{a}	91.51 ± 2.12^{ab}	95.20 ± 1.00^{a}	0.0114	*
36–40 weeks	81.41 ± 2.74^{b}	89.84 ± 2.97^{a}	88.06 ± 2.22^{ab}	90.07 ± 1.84^{a}	0.0184	*
28–40 weeks	84.91 ± 2.23^{b}	92.37 ± 1.75^{a}	90.40 ± 1.48^{ab}	93.97 ± 0.92^{a}	0.0038	**
Total feed consumption (g/hen)						
28–32 weeks	3064.78 ± 16.3^{a}	3107.13 ± 31.2^{a}	3096.92 ± 32.1^{a}	3108.60 ± 19.6^{a}	0.6034	NS
32–36 weeks	3041.76 ± 47.4^{a}	3100.17 ± 24.4^{a}	3093.74 ± 35.6^{a}	3107.30 ± 35.4^{a}	0.5817	NS
36–40 weeks	2994.73 ± 51.1^{a}	3075.36 ± 20.0^{a}	3073.32 ± 58.5^{a}	3085.75 ± 44.9^{a}	0.4851	NS
28–40 weeks	9101.27 ± 107.1^{a}	9282.66 ± 56.3^{a}	9263.98 ± 104.7^{a}	9301.64 ± 89.5^{a}	0.4038	NS
Feed conversion ratio (g feed/g egg)						
28–32 weeks	2.13 ± 0.06^{a}	1.95 ± 0.04^{ab}	2.02 ± 0.04^{ab}	1.90 ± 0.02^{b}	0.0021	**
32–36 weeks	2.08 ± 0.07^{a}	1.93 ± 0.05^{ab}	1.96 ± 0.05^{ab}	1.88 ± 0.02^{b}	0.0820	*
36–40 weeks	2.18 ± 0.08^{a}	1.99 ± 0.06^{ab}	2.02 ± 0.07^{ab}	1.95 ± 0.03^{b}	0.0160	*
28–40 weeks	2.12 ± 0.05^{a}	1.95 ± 0.04^{b}	2.00 ± 0.04^{b}	1.91 ± 0.02^{b}	0.0085	**

Means with different superscripts (a, b, c) in the same row are significantly different ($p < 0.05$).
*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; NS, not significant.

mented with propolis at the levels of 100 and 150 mg increased significantly compared to control group. Similarly, the findings of El-Neney et al. (2014) showed a significant increase in the total egg production and egg production percentage of laying hens fed diets supplemented with 200 or 300 mg propolis/kg as compared with those fed the control diet. On the other hand, Ozkok et al. (2013) reported that the egg production rate of laying hens fed diets supplemented with 0, 100, 200 and 400 mg/kg of propolis did not differ significantly ($p > 0.05$).

Also, our findings showed that the egg mass for treated groups was significantly heavier ($p < 0.05$) as compared with the control. The increase of egg mass for laying hens in the treated groups may be due to improving egg number, feed consumption and feed conversion compared with control.

These findings are in agreement with those of El-Neney et al. (2014), who indicated that egg mass was increased significantly by the addition of propolis to the diets of laying hens.

With regard to the feed conversion ratio, the obtained results showed that the hens in the control group increased significantly than those of the treated groups. This improvement in feed utilization for laying hens in the treated groups could be attributed to improved digestibility of the crude protein and the nutrient utilization due to the presence of high content of flavonoids and phenolic acids in propolis throughout a beneficial microbial environment in the gut. These results are in agreement with those of Buhatel et al. (1983), who revealed that pullets that received adequate levels of propolis in the diet remarkably improved feed conversion.

External and internal egg quality traits

External egg quality traits except eggshell weight was not affected by propolis treatments (Table 3). The improvement in eggshell weight could be attributed to the increase in calcium and phosphorus digestibility,

in addition to the high content of acid derivatives such as benzoic, 4-hydroxy-benzoic acid in propolis, which favour higher solubility of calcium and phosphorus salts in the diet, leading to increased absorption of the calcium (Haro et al., 2000 and Seven et al., 2011). These findings are in agreement with those of Ozkok et al. (2013), who found that egg weight for laying hens was not affected by the supplementation with propolis in the diets.

Internal egg quality traits (Haugh units, albumen height, yolk index and yolk weight) were significantly ($p \leq 0.05$) increased with propolis supplementation, whereas albumen weight and the percentages of albumen and yolk were not affected.

Haugh unit and yolk quality traits are important concern regarding the internal egg quality. Therefore, Haugh unit is a desirable characteristic of a numerical expression of albumen quality (Monira et al., 2003).

These results are in agreement with those of Galal et al. (2008) who found no significant difference among the treated groups in albumen and yolk percentages, whereas the eggs produced from laying hens fed diets that contained propolis at 50, 100 and 150 mg/kg levels recorded higher Haugh units as compared with control.

Similarly, the findings of Abou El-Naga (2014) indicated significantly increased eggshell percentage ($p < 0.05$) for laying hens fed diets that included 1% or 2% pollen than those of the control.

Blood constituents

Protein fractions

Data presented in Table 4 revealed that the total protein and globulin were significantly ($p < 0.05$) higher in the treated group than that of the control.

The increase in total protein could be attributed to improvement in synthesis and digestion of crude protein due to propolis administration.

Also, the increase in globulin level with increasing propolis levels could be attributed to the improved immunity of hens, through the better efficacy of the liver to synthesize enough globulins for immunological action. These results agree with those of El-Neney et al. (2014), who found that the means of plasma total protein, albumin and globulin in laying hens treated with propolis increased significantly than those of the control.

Enzymatic profile

As presented in Table 4, the findings showed that the laying hens fed diets supplemented with 250, 500 and 1000 mg propolis/kg diets had significantly ($p < 0.05$) lower AST and ALT concentrations as compared with those of the control group. These results indicated that the treatment with propolis is beneficial and safe through minimizing the liver functions and decreasing the harmful effect on its tissues. These results are in agreement with those obtained by El-Hanoun et al.

Table 3 Effect of supplementing the diets of laying hens with propolis on egg quality traits

Traits	Control	Propolis supplementation (mg/kg diet) 250	500	1000	p-Value	Sig.
External egg quality						
Specific gravity	1.10 ± 0.017^{a}	1.08 ± 0.001^{a}	1.09 ± 0.001^{a}	1.08 ± 0.001^{a}	0.4919	NS
Egg weight (g)	60.73 ± 0.83^{a}	62.05 ± 0.70^{a}	61.85 ± 0.82^{a}	62.86 ± 0.64^{a}	0.2546	NS
Shape index (%)	74.43 ± 0.72^{a}	75.50 ± 0.79^{a}	75.36 ± 1.07^{a}	75.53 ± 0.58^{a}	0.7389	NS
Surface area (g)	72.07 ± 0.69^{a}	73.19 ± 0.58^{a}	73.01 ± 0.68^{a}	73.87 ± 0.53^{a}	0.2427	NS
USSA (mg/cm²)	84.14 ± 0.34^{a}	84.70 ± 0.28^{a}	84.60 ± 0.33^{a}	85.03 ± 0.25^{a}	0.2268	NS
Shell weight (g)	7.94 ± 0.13^{b}	8.21 ± 0.12^{ab}	7.91 ± 0.12^{b}	8.39 ± 0.18^{a}	0.0469	*
Shell (%)	13.08 ± 0.16^{a}	13.26 ± 0.19^{a}	12.82 ± 0.17^{a}	13.37 ± 0.29^{a}	0.2827	NS
Internal egg quality						
Haugh unit (HU)	83.32 ± 1.05^{b}	85.58 ± 0.78^{ab}	85.25 ± 1.05^{ab}	86.32 ± 0.66^{a}	0.0102	*
Albumen height (mm)	7.08 ± 0.15^{b}	7.47 ± 0.13^{ab}	7.44 ± 0.17^{ab}	7.62 ± 0.11^{a}	0.0516	*
Albumen weight (g)	33.63 ± 0.66^{a}	33.28 ± 0.60^{a}	33.86 ± 0.71^{a}	33.87 ± 0.58^{a}	0.1808	NS
Albumen (%)	55.27 ± 0.53^{a}	53.56 ± 0.61^{a}	54.66 ± 0.77^{a}	53.79 ± 0.53^{a}	0.3276	NS
Yolk height (mm)	15.87 ± 0.25^{b}	16.76 ± 0.19^{a}	16.01 ± 0.30^{b}	16.84 ± 0.15^{a}	0.0037	**
Yolk diameter (mm)	41.29 ± 0.70^{a}	40.26 ± 0.61^{ab}	38.64 ± 0.52^{bc}	38.08 ± 0.79^{c}	0.0027	**
Yolk index (%)	38.91 ± 0.96^{c}	41.94 ± 0.71^{b}	41.75 ± 0.97^{b}	45.05 ± 1.10^{a}	0.0002	***
Yolk weight (g)	19.16 ± 0.30^{b}	20.56 ± 0.37^{a}	20.08 ± 0.47^{ab}	20.61 ± 0.32^{a}	0.0828	*
Yolk (%)	31.65 ± 0.46^{a}	33.18 ± 0.55^{a}	32.52 ± 0.71^{a}	32.84 ± 0.51^{a}	0.2618	NS

Means with different superscripts (a, b, c) in the same rows are significantly different ($p < 0.05$).
*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; NS, not significant.

Table 4 Effect of supplementing the diets of laying hens with propolis on some blood constituents, enzymatic and mineral profiles

Parameters	Propolis supplementation (mg/kg diet)				p-Value	Sig.
	Control	250	500	1000		
Total protein (g/dl)	5.77 ± 0.39^{b}	6.94 ± 0.32^{ab}	6.82 ± 0.45^{ab}	7.22 ± 0.36^{a}	0.066	*
Albumin (g/dl)	3.45 ± 0.24^{a}	3.52 ± 0.15^{a}	3.46 ± 0.23^{a}	3.62 ± 0.19^{a}	0.930	NS
Globulin (g/dl)	2.32 ± 0.37^{b}	3.42 ± 0.39^{ab}	3.36 ± 0.38^{ab}	3.60 ± 0.30^{a}	0.081	*
AST (U/l)	42.37 ± 1.94^{a}	33.0 ± 1.41^{b}	35.0 ± 1.55^{b}	32.25 ± 1.88^{b}	0.001	**
ALT (U/l)	12.50 ± 1.5^{a}	8.87 ± 0.69^{ab}	10.87 ± 1.5^{ab}	8.25 ± 1.08^{b}	0.082	*
Triglyceride (g/dl)	527.5 ± 32.9^{a}	498.4 ± 16.1^{a}	517.6 ± 29.3^{a}	478.7 ± 21.8^{a}	0.562	NS
Cholesterol (g/dl)	132.6 ± 2.04^{a}	117.2 ± 6.71^{b}	120.9 ± 5.9^{ab}	116.50 ± 3.2^{b}	0.093	*
Calcium (mg/dl)	22.01 ± 1.39^{a}	24.34 ± 0.80^{a}	23.52 ± 1.61^{a}	24.29 ± 2.90^{a}	0.7933	NS
Sodium (mg/dl)	23.70 ± 1.60^{a}	25.22 ± 2.87^{a}	24.11 ± 1.74^{a}	24.95 ± 2.05^{a}	0.9524	NS
Potassium (mg/dl)	24.67 ± 0.73^{a}	20.70 ± 1.96^{a}	21.36 ± 1.96^{a}	22.50 ± 1.12^{a}	0.3132	NS

Means with different superscripts (a, b, c) in the same rows are significantly different ($p < 0.05$).
*$p \leq 0.05$; **$p \leq 0.01$; NS, not significant.

Table 5 Effect of supplementing the diets of laying hens with propolis on haematological parameters

Parameters	Control	Propolis supplementation (mg/kg diet)			p-Value	Sig.
		250	500	1000		
Haemoglobin (g/dl)	9.81 ± 0.47^{b}	12.29 ± 1.08^{a}	11.11 ± 0.56^{ab}	12.86 ± 0.70^{a}	0.0340	*
RBC($\times 10^{6}/\mu$lit)	3.21 ± 0.18^{a}	3.42 ± 0.15^{a}	3.28 ± 0.12^{a}	3.46 ± 0.12^{a}	0.5742	NS
Basophil (%)	0.75 ± 0.36^{a}	0.50 ± 0.33^{a}	0.50 ± 0.33^{a}	0.25 ± 0.25^{a}	0.7500	NS
Eosinophil (%)	4.25 ± 0.88^{a}	3.75 ± 0.88^{a}	3.25 ± 0.75^{a}	3.00 ± 0.65^{a}	0.6967	NS
Monocyte %	1.25 ± 0.53^{a}	1.75 ± 0.25^{a}	1.50 ± 0.73^{a}	1.75 ± 0.25^{a}	0.8646	NS
Heterophil (%)	36.50 ± 1.34^{a}	30.50 ± 2.06^{b}	32.00 ± 2.30^{ab}	30.75 ± 1.60^{b}	0.0242	*
Lymphocyte (%)	57.25 ± 0.53^{b}	63.50 ± 1.80^{a}	62.75 ± 2.13^{a}	64.25 ± 1.38^{a}	0.0164	*
H/L ratio	63.81 ± 2.53^{a}	48.82 ± 4.16^{a}	52.26 ± 5.43^{a}	48.34 ± 3.36^{a}	0.0373	*

Means with different superscripts (a, b, c) in the same rows are significantly different ($p < 0.05$).
*$p \leq 0.05$; NS, not significant.

(2007) and Kamel et al. (2007), they reported that the treatment with propolis caused a significant ($p < 0.05$) decrease in serum AST and ALT levels compared to the control group. Similarly, the findings of Galal et al. (2008) showed that supplementing propolis at the levels of 100 and 150 mg propolis/kg diet significantly reduced AST and ALT concentration than those of the control group. Contrarily, Hashema et al. (2013) found no significant effect due to propolis supplementation on AST and ALT in broilers.

Referring to the cholesterol level, these findings showed that propolis in the diets at different levels significantly reduced cholesterol concentration in treated laying hens as compared with control group.

The decrease in the cholesterol level in the treated groups could be attributed to the fact that propolis might not be interfered with HDL-c synthesis but its mode of action might be induced by inhibiting cholesterol biosynthesis through inhibiting of HMG-CoA reductase, the rate-limiting enzyme that mediates the first step in cholesterol biosynthesis (Albokhadaim, 2015).

Mineral profiles

From Table 4, it could be noticed that the supplementation of propolis insignificantly affected to some extent plasma calcium and sodium as compared to the control group. These results are in agreement with those of Seven et al. (2009), who found that the concentrations of sodium and potassium were not significantly influenced by the addition of propolis. Similarly, the findings of Roodsari et al. (2004) showed no significant difference in blood calcium, sodium and potassium content of broiler chickens fed diets that contains 150, 450, 600 and 800 mg propolis/kg diet as compared to the control group. In contrast, Petruška et al. (2012) found a significant ($p < 0.05$) decrease in serum phosphorus and magnesium content of broiler chickens treated with propolis compared to control.

Blood haematology

Results presented in Table 5 indicated that supplementing laying hens with different propolis levels in the diets significantly increased ($p < 0.05$) the haemoglobin level (g/dl) than that of the control group. The

increase in haemoglobin level could be explained through the expected improvement in the digestive utilization of iron and the formation of ion, with higher efficiency in haemoglobin level (Haro et al., 2000), which is in agreement with the current findings.

Meanwhile, the improvement of RBCs count in the laying hens fed propolis could be attributed to its direct effect as a growth promoter on the haematopoietic tissues and due to the stimulating effect on the liver exhibiting an anabolic action, which favour the protein synthesis in addition to its preserving effect on the body protein from degeneration (James et al., 1994 and Bonomi et al., 2002).

With regard to white blood cells differentiation, the results showed that the supplementation of different levels of propolis significantly decreased the heterophils and increased the lymphocytes count as compared with the control group. The percentage of lymphocytes in hens fed diets supplemented with propolis may be due to its effects as antibacterial, antiviral and antifungal on their immunity system (Velikova et al., 2000; El-Bassuony, 2009).

In chickens, the heterophils are phagocytic cells whose main function is the protection against invading micro-organisms, whereas the primary function of lymphocytes is involving in cell-mediated and humeral immunity. Heterophils increase and lymphocytes decrease when they are stressed, so that the ratio between them is a good index of response to a stressor (Gross and Siegel, 1985).

In accordance with H/L ratio, the achieved results showed that the propolis supplementation at the levels of 250, 500 and 1000 mg/kg diet significantly ($p < 0.05$) decreased the H/L ratio in laying hens. The H/L ratio is a recognized measure for stress in birds (Davison et al., 1983; and Maxwell, 1993), which has become a valuable tool in stress research, especially when combined with the convenience and repeatability of automated blood cell counts.

Table 6 Economical efficiency of laying hens affected by propolis supplementation

Items	Propolis supplementation (mg/kg diet)			
	Control	250	500	1000
Price of propolis (L.E)	0	2.3205	4.632	9.301
Total FI hen (kg)	9.101	9.282	9.264	9.301
Total feed cost/hen (L.E)	25.94	26.45	26.40	26.51
Total cost (propolis + feed)	25.94	28.77	31.03	35.81
Total EN/hen	71.22	77.59	75.94	78.94
Price/egg (L.E)	0.75	0.75	0.75	0.75
Total price of egg (L.E)	53.41	58.19	56.95	59.20
Net revenue per hen	27.47	29.42	25.92	23.39
Relative (REE)	100	107.1	94.32	85.15

Feed cost/kg = £2.85 L.E.

On the other hand, analysis of differential leucocyte percentages indicated no significant differences in the percentage of eosinophils, monocytes and basophiles in hens fed diets including propolis than those of the control group.

From data presented in Table 6, it could be noticed that supplementing laying hens' diet with 250 mg/kg of propolis was more economical and cheaper than control and other levels of propolis.

Conclusion

These results could be summarized as follows:

(i) Based on these data, it can be recommended to use propolis at 250, 500 and 1000 mg/kg diet as a good natural product to improve the egg production and hematological parameters of laying hens.

(ii) The treated hens with propolis had significantly ($p < 0.05$) increased egg mass, egg production rate, shell weight, HU, albumen height and yolk index, TP, Glb and lymphocyte and decreased AST, ALT, cholesterol, heterophils and H/L ratio.

(iii) Using propolis at 250 mg/kg diet is more economical, especially for small producers.

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