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THE EFFECT OF SUPPLEMENTING DIET WITH PROPOLIS ON BANDARAH LAYING HENS' PERFORMANCE

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ABSTRACT: The aim of this study was to evaluate the influence of supplementing diet with propolis on Bandarah laying hens' performance. A total of 120 laying hens and 32 cocks at 30 weeks of age were randomly distributed among four treatments groups with 3 replicates each (10 females and 1 male/replicate) were housed in floor pens (2.5x1.5x2.5m).Birds in group 1 were fed a basal diet and considered as control group, while those in groups 2, 3 and 4 were fed on the same basal diet supplemented with 150,300 and 450 mg propolis/kg diet during experimental period (30-42 weeks of age). Results indicated that egg weight, egg production % and egg mass for propolis treatments were significantly ($p<0.01$) increased than those fed control diet. Feed consumption was not affected by supplemental propolis, while feed conversion ratio was significantly improved compared with control group. Shell thickness, Haugh unit score and egg yolk % significantly improved for hens fed diet supplemented with propolis compared with control group. However, egg shell %, shape index, yolk index, albumen % and yolk color score were not affected by propolis supplementation. In accordance with hematological parameters, addition of propolis at different levels significantly ($p<0.01$) increased Hb, PCV, RBC, WBC, lymphocytes count while heterophils count significantly ($p<0.01$) decreased. There was significant increase in plasma total protein, globulin, IgG and IgM with increasing propolis level. Lipid profile, Liver and kidney function significantly ($p<0.01$) improved for propolis treatments. Significant decrease was observed in plasma lipid peroxidation based on MDA levels in treated groups compared with control group also, results showed significant increase in antioxidants enzymes (TAC and SOD) for the groups supplied with propolis. Moreover, supplementation diets with propolis at different levels significantly improved semen quality, fertility and hatchability percentages compared with control. In conclusion, the results indicated that supplementation of propolis to Bandarah chickens diets significantly improved productive, reproductive, physiological, immunological and anti-oxidative status.

Key Words: Laying hens- propolis- egg production- blood constituents- immunity.

INTRODUCTION

Supplementation of natural components in poultry ration are now widely distributed in the world. These components are served as growth promoting, which are healthful and help to improve the production performance of animal and poultry without any harmful effect. 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 (Kwiecien and Winiarska-Mieczan, 2009).

Propolis is a natural resin of complex chemical composition and its principal role to maintain an antiseptic environment in the bee hive and to enable the bee colony health. It is a substance obtained from several parts of the plant such as buds, floral buds, leaves, branches and barks, in which the composition varies according to the flora of each region visited by the bee. More than 300 constituents have been identified in different propolis samples (Turkez et al., 2010); mostly include a mixture of polyphenols, flavonoid aglycones, phenolic acid and their esters, and phenolic aldehydes and ketones, terpenes, sterols, vitamins and amino acids **(**Khalil, 2006). Several biological properties such as antibacterial and antifungal (Mohammadzadeh et al., 2007), antiviral (Amoros et al., 1992), anti-inflammatory (Dobrowolski et al., 1991) and immunomodulatory (Missima and Sforcin, 2008) may be attributed to the variety of chemical compounds identified in propolis.The biological potential of propolis could be due to a synergism that occurs among their constituents.

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. Dietary supplementation of laying hens exposed to heat stress with propolis (5 g/kg) can attenuate heat stress-induced oxidative damage and increase growth performance and digestibility, improve eggshell thickness and egg weight **(**Tatli-Seven, 2008; and Tatli-Seven et al., 2009). A similar trend was also observed by Abdel-Kareem and El-Sheikh (2015) who found that the averages of egg numbers and egg production rate for hens treated with propolis at 250 and 1000 mg/kg diet significantly ($p<0.05$) increased than those of the control group.

With regard to the propolis supplementation on plasma cholesterol, El-Neney et al. (2014) showed that plasma cholesterol was significantly reduced ($p<0.05$) in Dokki 4 laying hens fed propolis compared with control. Also, they reported that plasma total protein, albumin and globulin were significantly lower in 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 in RBC and WBC, Hb and lymphocytes. Cetin et al. (2010) demonstrated that addition of propolis at 3 g/kg in the laying hens' diet resulted in significant increases in the serum IgG and IgM levels and erythrocyte count.

Recently propolis is the most important dietary supplement as antioxidant compound because of their anti- stress effects (Tatli Seven, 2008; and Seven et al., 2011). Furthermore, propolis can relieve the adverse effects of lipid peroxidation and free radical formation. Adding propolis at 50 mg/kg feed for the entire period of rearing broiler chickens, protected the liver of birds against pathological lesions **(**Babinska et al., 2012). The antioxidant activity of propolis is mainly attributed to its flavonoid contents, such as quercetin, flavones, isoflavones, flavonones, anthocyanins, catechins and isocatechins (Alves and Kubota, 2013) that are capable of

scavenging free radicals and thereby protection against lipid peroxidation. It has been demonstrated that propolis provides protection against infertility by improving sperm production, motility, count and quality increasing the process of steroidogenesis and hence testosterone production (Yousef and Salama, 2009).

The study aim to evaluate the effects of Propolis supplementation to the diet of Bandarah chickens on productive and reproductive performance and immune response.

MATERIALS AND METHODS

The present experiment was carried out at El-Sabahia Poultry Research Station, Animal Production Research Institute, Agricultural Research Center, Egypt. A total of one hundred and twenty laying hens and thirty two cocks of Bandarah strain at 30 weeks of age were used in this experiment. Birds were distributed at random into four treatments groups and each group was subdivided into 3 replicates each with (10 females and 1 male/replicate) and housed in floor pens 2.5 x 1.5 x 2.5 m. Twenty cocks were housed individually in single cages and distributed randomly in the same four treatments groups (5 in each one) for semen evaluations.

Birds in all groups were kept under the same environmental and managerial conditions. Feed and water were supplied *ad libitum* throughout the experimental period which ended at 42 wks of age. Artificial lighting was used to provide birds 17 hrs lighting daily. The basal diet (control) was formulated to meet nutrient requirements of chickens. The composition of the basal diet is given in Table (1). The birds were fed basal diet (T1, control) or basal diet supplemented with 150, 300 and 450 mg propolis /kg diet for T2, T3 and T4, respectively.

Productive parameters:

Egg weight (g) and egg number were recorded daily. Egg mass was calculated by multiplying egg number by average egg weight per hen (g/h/d). Feed conversion was calculated as the amount of feed consumed (g) required to produce a unit (g) of egg mass (g feed/g egg). Egg quality was measured, in which 15 eggs were randomly taken from each treatment. Eggs were individually broken out, shape index, albumen and yolk index values were measured according to Sauter et al. (1951) as follow:

- Shape index (%) = (width/length) x 100
- Yolk index (%) = (height/diameter) x 100
- Egg shell thickness, without inner membranes was measured (mm) with the micrometer. The height of thick albumen (H) and the egg weight (W) were used to calculate Haugh Units (HU) from the formula of Haugh (1937): HU = 100 log (H + 7.57 - 1.7 $W^{0.37}$), where H= thick albumen height, W= egg weight.

Blood constituents:

At the end of the study, blood samples were randomly taken from 10 hens from each treated group in heparinized tube from the brachial wing vein. A portion of the fresh blood was used to measure the white blood cells (WBCs), different subclasses of WBC's (lymphocytes and heterophils), red blood cells (RBCs), hemoglobin (Hb) and packed cell volume (PCV). Plasma was obtained from the blood samples by centrifugation for 15 min. at 3000 rpm and was stored at -20 ˚C until the time of analysis. Plasma calcium, phosphorus, total protein, albumin, total lipids, cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), glucose, and urea, creatinine, alkaline phosphatase (ALP), alanine aminotransferase (ALT), and asparatate aminotransferase (AST) were determined by spectrophotometrically using available commercial Kits. Plasma immunoglobulin, IgG and IgM were determined using the method of Leslie and Frank (1989). Total antioxidants capacity (TAC), Malondialdehyde (MDA) and Superoxide dismutase (SOD) activity were

calorimetrically determined using commercial Kits.

Semen evaluation:

At 34 weeks of age, semen samples were collected from cocks of each treatment once weekly by abdominal massage technique. Some semen physical properties such as ejaculate volume (ml), forward motility (%), live sperm (%) and sperm concentration were determined.

Fertility and Hatchability percentages:

For evaluating egg fertility and hatchability three hatches of eggs were made every 4 weeks of the experimental period. A total of 880 hatched eggs representing the four experimental dietary groups were incubated in Egyptian-made incubator at 37.8 C and 55% RH during incubation and transferred to hatcher operated at 37.2 C and 65% RH. Egg fertility and hatchability for set and fertile eggs were determined. Weights of healthy chicks were also recorded.

Economic efficiency:

The total feed cost (L.E/hen) at the end of the experiment for each treatment was calculated depending on the local market prices of the ingredients used for formulating the experimental diet. Economic efficiency (EE) and relative economic efficiency (REE) were calculated using the following equation:

EE = Net return LE /Total feed cost LE.

Statistical analysis:

Data were statistically analyzed according to SAS program (SAS, 2004) using GLM Procedure. All the data were subjected to one way analysis of variance model. Mean differences were tested by Duncan's multiple range (Duncan, 1955).

RESULTS AND DISCUSSION

Productive performance

Egg production parameters, feed consumption and feed conversion ratio of laying hens fed diets supplemented with different levels of propolis are summarized in Table (2). Results revealed that egg weight (EW) and egg mass (EM) were significantly ($p<0.01$) increased by supplementation of propolis (150, 300 and 450 mg/ kg diet) compared with control group. Supplementation of graded levels of propolis (150, 300 and 450 mg/kg diet) significant increased ($p<0.01$) egg weight (EW) by 2.60, 5.66 and 5.66 % and egg production (EP) by 12.14, 33.00 and 37.98 %, respectively compared with control group. However, both 300 and 450 mg propolis supplementation were equally effective on egg weight. The improvement in EW and EP for treated groups may be attributed to propolis pronounced contents of some digestive enzymes (glucose oxidase, catalase and peroxidase) and the essential aromatic oils which improved digestibility of the different nutrients (Khojasteh and Shivazad, 2006).

Results are in agreement with those reported by Galal et al. (2008) who found that laying hens fed diet supplemented with propolis (100 and 150 mg/kg diet) significantly increased egg production rate and egg weight compared to control group. Similarly, El-Neney et al. (2014) showed a significant increase in the egg production percentage and egg weight of laying hens fed diets supplemented with 200 or 300 mg propolis / kg compared with those fed the control diet. Also, Abdel-Kareem and El-Sheikh (2015), showed that propolis supplementation to the layer diets at 250, 500 and 1000 mg improved egg production while the egg weight was not influenced by the treatments.

Increasing both of EW and EP reflected on significant increasing of egg mass (EM), whereas the hens fed diet containing 150, 300 and 450 mg propolis produced significantly heaviest egg mass by 15.11,40.57 and 45.84%, respectively compared with control group. Similar results were confirmed by (El-Neney et al. 2014; and Abdel-Kareem and El-Sheikh, 2015) who indicated that egg mass was increased significantly by addition of propolis to the laying hen diets.

Regarding the feed consumption, the result showed that the propolis supplementation to laying hen diets had no significant effect on the amount of feed consumed. This result is consistent with those previously reported by Ozkok et al. (2013) who found that feed intake was not influenced for laying hens fed diets supplemented with 100, 200 and 400 mg propolis / kg. This result does not agree with the finding of Guclu-Kocaoglu (2010) who noticed that laying hens fed diets containing 0.5, 1, 3 and 6 g propolis / kg significantly increased feed consumption than those of the control.

With respect to the feed conversion ratio (FCR), the results showed that FCR significantly improved for laying hens fed diets supplemented with propolis by 12.62, 28.06 and 30.66% compared with control group.

This improvement in feed utilization for laying hens in the treated groups could be attributed to improved digestibility of the crude protein and the other nutrients due to the presence of high content of flavonoids and phenolic acids in propolis which improve a beneficial microbial environment in the gut. This result is in harmony with finding of Galal et al. (2008) who revealed that laying hens that received 100 and 150 mg propolis /kg diet significantly improved feed conversion. Similar results were confirmed by Abdel-Kareem and El-Sheikh (2015) who indicated that feed conversion was improved significantly by addition of propolis to layer diets at levels 250, 500 and 1000 mg Propolis / kg diet.

Egg quality traits:

The effect of supplemental propolis on egg quality traits of Bandarah laying hens are presented in Table (3). Egg shell, shape index, egg yolk index, albumen % and yolk color score were not affected by propolis supplementation. These findings are in agreement with those of **Tatli-Seven (2008)** who found that supplementation of propolis at the levels of 2 and 5 g to layer diets did not affect on egg shell and egg shape index %. On the other hand, El-Neney et al. (2014) reported that the laying hens fed diets supplemented with 100, 200 and 300 mg propolis / kg significantly increased the egg shape index %. The present study indicated that shell thickness, Haugh unit score (HU) and egg yolk % significantly improved for laying hen fed diet supplemented with propolis by 9.38, 15.63 and 15.63%, respectively for egg shell thickness, 2.00, 4.89 and 5.36%, respectively for HU compared with control group. These results are in agreement with Tatli-Seven (2008) who observed that supplemental propolis significantly increased ($p<0.01$) the egg shell thickness compared to control group. The improvement in egg shell thickness could be attributed to the increase in calcium and phosphorus digestibility, also may be due to the high content of acid derivatives such as benzoic, 4-hydroxy-benzoic acid in propolis, which favor higher solubility of calcium and phosphorus salts in the diet, leading to increasing absorption of the calcium (Haro et al., 2000; and Seven et al., 2011). Improvement of HU is characteristic for egg quality (Monira et al., 2003). This result is in agreement with Galal et al. (2008) who indicated that the eggs produced from laying hens fed diets contained propolis at 50, 100 and 150 mg/kg recorded higher Haugh units as compared to control. Similarly, Abdel-Kareem and El-Sheikh (2015) showed significantly increased ($p<0.5$) Haugh units for laying hens fed diets that included 250, 500 and 1000 mg propolis / kg than those of the control. Inversely, Ozkok et al. (2013) found that the addition of propolis to laying hen ration at doses of 100, 200 and 400 mg/kg did not affect Haugh unit values.

Result of albumen percentage, yolk index and yolk color score are in harmony with the finding of El-Neney et al. (2014) who indicated that yolk percentage for

laying hens was increased significantly by the addition of propolis to the laying hen diets. Similarly, Wang et al. (2007) cleared that bee pollen supplementation (1.5 %) increased egg yolk by 6.89 % compared to the control group.

Biochemical parameters:

Hematological parameters:

Results of Table (4) indicated that Hb, RBCs, PCV and WBCs were significantly ($p<0.01$) increased with addition of propolis. However, supplementation of propolis at 300 and 450 mg were equally effective and had the significant highest values of Hb, RBCs, PCV and WBCs compare with control group. Our findings were supported by Galal et al. (2008) who found that addition of propolis at 100 and 150 mg/kg layer diet significant increase heamatocrit level. Also, Cetin et al. (2010) reported that supplementation layer diets with propolis at 3g/kg significantly increased RBCs and Hb levels. Significant elevations of the erythrocytes numbers reported in the present study may be due to propolis have a stimulatory effect on synthesis of these cells from bone marrow. Similar results were confirmed by Orsolic and Basic (2005). The improvement in Hb value resulted from the addition of propolis could be explained by assuming that propolis improves the digestive utilization of iron and the regeneration efficiency of hemoglobin (Haro et al., 2000).

Immune response:

White blood cells differential count for laying hens fed different levels of propolis are presented in fig (1). Result indicated that lymphocytes count (L) was significant increase ($p<0.01$) in treated groups, while, propolis supplementation significantly decreased ($p<0.05$) the heterophils count (H). Supplementation layer diet with propolis (150, 300 and 450 mg/kg) significantly decreased heterophils / lymphocytes ratio (H/L ratio) by 10.52, 24.56 and 24.56 %, less than the control group, respectively. However, propolis levels at 300 and 450 mg had equal effect in H/L ratio.

Figure (2) shown that supplementation layer diets with all levels of propolis caused a significant increase in plasma IgG and IgM values compare with the control group. These results are in agreement with Cetin et al. (2010) who reported that addition of propolis (3g/kg diet) resulted in significant increase in serum IgG and IgM levels in laying hens. Freitas et al. (2011) demonstrated that adding propolis to laying hens rations (50mg/kg) increased the production of IgG specific to SRBC and decreased heterophils count and H/L ratio. Also, El-Neney et al. (2014) showed that propolis supplementation to the ration of laying hens at levels (100, 200 and 300 mg/kg) increased leukocytes count, lymphocytes and plasma IgG and IgM. The improvement in immunological status may be due to propolis has more than 160 constituents that have several biological and pharmacological properties such as antimicrobial, anti-inflammatory, immunomodularity and antioxidant effects (Pari and Gnana-Soundari, 2006; Kanbur et al., 2009; Newairy et al., 2009; and Orsatti et al., 2010). Moreover, Artepillin C which is one of propolis components has been described to activate the immune system by increasing phagocytic activity as well as number of lymphocytes (Kimoto et al., 1998).

Table (5) shows the effect of dietary propolis supplementation on plasma total protein, albumin and globulin of Bandarah laying hens. With respect to plasma total protein and globulin, it could be speculated that the propolis supplemental significantly increased both of them compared with control group and this increase was in a level-dependent manner. While, no significant differences were observed in plasma albumin among the experimental groups. The increase in total protein and globulin with propolis addition may be due to the increase in protein synthesis.

According to, Scheller et al. (1977) and Gabrys et al. (1986) propolis stimulated mammalian tissue regeneration, as it caused strong activation of mitosis of cells cultured in vitro and it enhanced protein biosynthesis. Increased globulin concentration with increased propolis inclusion which was observed in the present study may be an indication of increased immunity in the laying hens since the liver will be able to synthesize enough globulins for immunologic action as mentioned by Sunmonu and Oloyede (2007). These findings were confirmed upon examination of propolis on laying hens (Galal et al., 2008; and El-Neney et al., 2014). Abdel-Kareem and El-Sheikh (2015) found that propolis supplementation (250, 500 and 1000 mg / kg diet) increased total protein and globulin for laying hens.

Propolis supplementation numerically increased blood plasma calcium and phosphorus concentrations than control, but this increase was not significant (Table 5). This result is harmony with finding of Galal et al. (2008) who indicated that propolis supplementation at levels 50, 100 and 150 mg /kg diet increased plasma calcium and phosphorus compared to control group in laying hens. The improving in digestibility of calcium and phosphorus may be due to the acid derivatives (such as benzoic,4-hydroxy-benzoic, etc…) which are found in propolis (Foucher, 1982), and that favor the solubility of calcium and phosphorus salts in the diet, thus increasing the absorption of calcium.

Results of Table (5) indicted that lipid profile (total lipids, cholesterol, LDL and HDL) was a significant improved ($p<0.01$) by adding propolis to layer diets. Whereas, total lipids, cholesterol and LDL were significantly decreased while, HDL was significant increased compared with control diet. These results are in agreement with those reported by Galal et al. (2008) who observed that supplementation of propolis at 100 and 150 mg/kg layer diet decreased cholesterol statues. Also, El-Neney et al. (2014) mentioned that supplementation layer diets with different levels of propolis (100, 150 and 300 mg/kg diet) significantly decreased total plasma and yolk total lipids and cholesterol. Results of plasma lipids revealed that propolis has a decreasing effect on cholesterol and LDL which may be attributed to the presence of flavonoids, steroids, phenolic acids and their esters among propolis constituents (Hegazi et al., 2004; Talas and Gulhan 2009). These compounds may affect directly lipid metabolism leading to decrease of cholesterol and triglycrides in blood (Eraslan et al., 2007). Additionally, Alves et al. (2008) reported that the hypocholesterolemic effect of propolis may be a result of a direct effect on the liver or an indirect effect through thyroid hormones which affect reactions in almost all the pathways of lipid metabolism.

Table (5) shows that laying hens fed diets supplemented with propolis at any inclusion level had no significant effect on plasma glucose concentration compared with the control. Similar results were confirmed by Biavatti et al. (2003). Seven et al. (2010) reported that blood glucose concentration was not affected by supplementation vitamin C and propolis in the diet for broilers exposed to oxidative stress. On the other hand, these results are in contrast with the other findings of Hashem et al. (2013) who recorded that propolis supplementation to rabbit bucks resulted in a significant increase in blood plasma glucose.

Kidney function and liver enzymes:

Propolis supplementation significantly improved kidney function whereas, plasma urea and creatinine were significantly decreased ($p<0.01$) by 11.o, 23.31 and 24.13 % and 34.90, 46.35 and 50.00 %, respectively for the groups supplied with 150,300 and 450 mg propolis /kg diet. However, both 300 and 450 mg propolis supplementation has the same potent effect for protecting kidney

function. These results are in agreement with findings by Attia et al. (2014) who found that plasma urea and creatinine were decreased by dietary supplementation of 300 mg propolis/kg diet compared with control group in broiler chickens. Likewise, our findings were confirmed upon examination of propolis on rabbits (El-Hanoun et al., 2007; and Attia et al., 2015). The role of propolis in protect kidney discussed by Sun et al. (2000) who concluded that propolis exerts its antioxidative effect where it is assumed to accumulate, such as on the kidney, where it is excreted, and on the gastrointestinal tract, where propolis influence these tissues even from the outside of the cell.

Results indicated that liver function was significantly affected by treatment Table (6). Whereas, serum transaminases (AST and ALT) and ALP activity were significantly decrease ($p<0.01$) for treated groups supplied with propoils compared with the control group. However, supplied propolis by 300 and 450 mg/kg had the same effect on liver enzymes. The improvement in liver and kidney function may be attributed to higher biological activity and nutritive values contents in propolis, which could prevent lipid peroxidation. The present results concerning the decreasing of the serum transaminases activities are in agreement with the results obtained by Galal et al. (2008) who found that plasma liver enzymes (AST and ALT) were significantly reduced when laying hens fed diet containing 100 and 150 mg propolis/kg diet. Similarly, Abdel-Kareem and El-Sheikh (2015) noticed that AST and ALT decreased significantly with increasing propolis at levels 250, 500 and 1000 mg/kg laying diet. In another study on Japanese quail, Eid et al. (2014) reported that adding propolis to drinking water (8 and 16 ml propolis extract/L drinking water) significantly decreased ($p<0.010$) ALT activity compared with control while, AST activity not affected. The improvement in liver and kidney function may be attributed to higher biological activity and nutritive values contents in propolis, which could prevent lipid peroxidation.

Antioxidants activities and lipid peroxidation:

Fig (3) showed that there was a significant effect ($p<0.01$) of adding propolis to layer diets at different levels on plasma total antioxidants capacity (TAC), superoxide dismutase (SOD) and malondialdehyde (MDA) activities. This effect was in a level-dependent manner, whereas, plasma TAC value was significantly boosted by 22.03, 45.76 and 55.93 % compare with control group. The same trend was shown in the plasma SOD activity, which showed a significant ($p<0.01$) increase by increasing propolis level. Supplementation of propolis to laying hen rations significantly decrease ($p<0.01$) plasma MDA concentration compared with control group. This result was in harmony since, increasing antioxidant enzymes (TAC and SOD) for the groups supplied with propolis is an indicator for protection against oxidative status which decreases MDA level in treated groups. These findings are confirmed with those previously reported by Tatli-Seven (2008) who observed that dietary supplementation of laying hens with vitamin C (250 mg/kg diet) and propolis (2 and 5 g of ethanol extracted propolis/kg) could attenuate heat stress-induced oxidative damage by increasing TAC activity which resulted in decreasing MDA level. El-Neney et al. (2014) found that supplementation diet with propolis (100, 200 and 3oo mg/kg) boosted layer plasma TAC values. According to De Sa' et al. (2013) propolis could be considered as a promising antioxidant product due to three many reasons: 1- it contributes to protect membrane lipids from H_2O_2 stress, 2- in response to an O_2-stress mediated by menadion, propolis acts maintaining the redox status by scavenging reactive

oxygen species (ROS) and 3- it activates Cu/Zn-superoxide dismutase. The antioxidant activity of propolis may be due to the ability of phenolic compounds to donate hydrogen ions that can attack the free radicals to prevent the oxidation reactions in the cell (El-Sohaimy and Masry, 2014).

Semen traits:

Results of semen traits (ejaculate volume, sperm concentration, sperm abnormality, sperm motility and sperm motility index) are illustrated in Table (7). Propolis supplementation to cocks diets significantly improved semen quality. Whereas, a significant increase ($p<0.01$) was observed in sperm concentration, sperm motility and sperm motility index due to supplementation of propolis in cocks' rations and this effect was in a level-dependent manner. Moreover, the opposite trend was shown in the sperm abnormality which showed a significant decrease ($p<0.01$) in the groups supplied with150, 300 and 450 mg propolis/ kg diet by 9.96, 24.87 and 25.17 %, respectively less than the control value. On the other hand, no significant differences were observed among treated groups for ejaculate volume. These results are in agreement with the findings of El-Neney et al. (2014) for cocks, Yousef et al. (2010) for rabbits. Moreover, the improvement in semen quality reported in the present study may be attributed to the antioxidant properties of flavonoids found in propolis which improve sperm morphology and protecting the sperm membrane (Syazana et al., 2011).

Fertility and hatchability percentages:

The results in Table (8) showed significant increase ($p<0.01$) in fertility, hatchability percentages and chick weight at hatch for treated groups compared to control and this increase was in a level-dependent manner. The improvement in fertility and hatchability % resulted from the addition of propolis are in agreement with the results of El-Neney et al. (2014) who noted that, supplementation layer diets with propolis levels (100, 200 and 300 mg/kg) significantly improved fertility and hatchability percentages compared with control. The improvement in fertility may be attributed to propolis provides protection against infertility by increasing the process of sterioidogenesis and hence testosterone production (Yousef and Salama, 2009). From our results, adding propolis to layer diets increased egg weight and improved in egg quality may be reflected on increasing chick weight. At hatch.

Economical efficiency:

Data illustrated in Table (9) showed that laying hens' diet supplemented with propolis gave more economic efficiency than the control group and increased net revenue. Moreover, supplied layer diets with 300 mg propolis /kg was more economical than other levels of propolis.

CONCLUSION

The results of the present study concluded that supplementation of propolis at different levels to layer diets were efficient in improving the productive, reproductive performance traits, egg quality, semen quality, fertility and hatchability percentages and has beneficial effects on physiological, immunological and anti-oxidative status. The best results in most studied traits were recorded for diets contained 300 and 450 mg but 300 mg was more economical than other levels of propolis.

Table (1): Composition* and the nutritive value of the basal diets.

Ingredients	%	Calculated Composition	
yellow Corn	63.55	Crude Protein, %	16.50
Soybean M. (CP44%)	25.10	ME, Kcal/kg	2700
Premix**	0.30	Crud fiber, %	2.60
NaCl	0.40	Ca, %	3.50
Di. Ca. phosphate.	1.45	P(va) , %	0.40
Limestone	8.10	Ly, %	0.89
Mineral supplementations	1.00	Meth, %	0.36
DL-methionine (Meth)	0.10	Total sulpher amino acids %	0.66
Total	100		

*****As recommendation of Anim. Prod. Res. Inst., Agric Res. Center, Minis. of Agric., (2001). ****** Composition of premix in 3 kg is: Vit. A 10,000,000 IU, Vit. D_3 2,000,000; Vit. E 10,000 mg, Vit. K_3 1,000 mg, Vit. B_1 1,000 mg, Vit. B_2 4,000 mg, Vit. B_6 1,500 mg, Vit. B_{12} 10 mg; Niacin 20,000 mg; Pantotenic acid 10,000 mg, Folic acid 1,000 mg, Biotin 50 mg, Choline chloride 500, 000 mg, Cu 3,000 mg, Iodine 300 mg, Fe 30,000 mg; Mn 40,000 mg, Zn 45,000 mg, Selenium 100 mg.

Table (2): Productive performance of Bandarah laying hens fed diets supplemented with different levels of propolis.

Parameters	Propolis levels (mg/kg diet)				Pooled SEM	Sig.
	0	150	300	450		
Egg weight (g)	48.2 ^{c}	49.47^{b}	50.93 ^{a}	50.93 ^{a}	0.35	**
Egg production (%)	44.23 ^{c}	49.60 ^{b}	58.83 ^{a}	61.03 ^{a}	2.09	**
Egg mass (g/h/d)	21.32 ^{c}	24.54 ^{b}	29.97 ^{a}	31.09 ^{a}	1.22	**
Feed consumption(g/h/d)	118.20	118.83	119.33	119.60	0.41	NS
Feed conversion ratio(g feed/ g egg)	4.99 ^{a}	4.36 ^{b}	3.59 ^{c}	3.46 ^{c}	0.19	**

a, b, c Means in the same row with different superscripts, differ significantly ($p<0.05$). Sig.= Significance, **($p<0.01$). NS = Not Significant. SEM = standard error mean.

Table (3): Egg quality of Bandarah laying hens fed diets supplemented with different levels of propolis.

Parameters	Propolis levels (mg/kg diet)				Pooled SEM	Sig.
	0	150	300	450		
Shell (%)	11.25	11.07	11.21	11.28	0.28	NS
Shell thickness (mm)	0.32 ^{c}	0.35 ^{b}	0.37 ^{a}	0.37 ^{a}	0.00	**
Haugh unit score	83.58 ^{c}	85.25 ^{b}	87.67 ^{a}	88.06 ^{a}	1.14	**
Shape index (%)	78.43	77.49	78.56	77.79	0.39	NS
Yolk color score	7.55	7.42	7.46	7.55	0.07	NS
Yolk index (%)	46.94	47.36	47.82	47.65	0.42	NS
Yolk (%)	31.84 ^{b}	32.12 ^{b}	33.56 ^{a}	33.60 ^{a}	0.40	*
Albumen (%)	56.91	56.81	55.23	55.12	0.58	NS

a, b, c Means in the same row with different superscripts, differ significantly ($p<0.05$). Sig.= Significance, *($p<0.05$), **($p<0.01$). NS = Not Significant. SEM = standard error mean.

Table (4): Hematological parameters of Bandarah laying hens fed diets supplemented with different levels of propolis.

Parameters	Propolis levels (mg/kg diet)				Pooled SEM	Sig.
	0	150	300	450		
Hb (g/dl)	9.55 ^{c}	10.77^{b}	11.67 ^{a}	11.99 ^{a}	o.16	**
RBCs (10^6/mm^3)	2.34 ^{c}	3.19 ^{b}	4.09 ^{a}	4.09 ^{a}	0.13	**
PCV (%)	27.78 ^{c}	29.94 ^{b}	33.58 ^{a}	33.94 ^{a}	0.44	**
WBCs (10^3/mm^3)	5.11 ^{c}	6.10 ^{b}	7.18 ^{a}	7.56 ^{a}	0.16	**

Hb= hemoglobin; RBC= red blood cells; PCV= packed cell volume; WBC= white blood cells. a, b, c Means in the same row with different superscripts, differ significantly ($p<0.05$). Sig.= Significance, **($p<0.01$). NS = Not Significant. SEM = standard error mean.

Table (5): Some blood constituents of Bandarah laying hens fed diets supplemented with different levels of propolis.

Parameters	Propolis levels (mg/kg diet)				Pooled SEM	Sig.
	0	150	300	450		
Total protein(g/dl)	4.86 ^{c}	5.32 ^{b}	6.14 ^{a}	6.41 ^{a}	0.12	**
Albumin (g/dl)	3.45	3.41	3.47	3.57	0.03	NS
Globulin (g/dl)	1.41 ^{c}	1.91 ^{b}	2.67 ^{a}	2.84 ^{a}	0.11	**
Calcium (mg/dl)	16.61	16.71	17.21	17.5	0.13	NS
Phosphorus(mg/dl)	6.28	6.45	6.41	6.48	0.07	NS
Total lipids (mg/dl)	451.38 ^{a}	401 ^{b}	354.63 ^{c}	347.40 ^{c}	8.24	**
Cholesterol(mg/dl)	130.63 ^{a}	126.13 ^{b}	120.88 ^{c}	116.80 ^{d}	0.98	**
HDL(mg/dl)	28.88 ^{c}	41.50 ^{b}	55.38 ^{a}	55.40 ^{a}	2.12	**
LDL (mg/dl)	96.63 ^{a}	86.75 ^{b}	72.25 ^{c}	71 ^{c}	2.02	**
Glucose (mg/dl)	193.75	194.38	192.13	196.2	1.95	NS

HDL= High density lipoprotein; LDL= Low density lipoprotein. a, b, c, Means in the same row with different superscripts, differ significantly ($p<0.05$). Sig.= Significance, ** ($p<0.01$). NS = Not Significant. SEM = standard error mean.

Table (6): Kidney function and liver enzymes of Bandarah laying hens fed diets supplemented with different levels of propolis.

Propolis levels (mg/kg diet)	Kidney function		Liver enzymes		
	Urea (mg/dl)	Creatinine (mg/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)
0	26.73 ^{a}	1.92 ^{a}	93.93 ^{a}	43.16 ^{a}	150.13 ^{a}
150	23.79 ^{b}	1.25 ^{b}	90.54 ^{b}	39.27 ^{b}	144.63 ^{b}
300	20.5 ^{c}	1.03 ^{c}	88.16 ^{c}	35.87 ^{c}	138.63 ^{c}
450	20.28 ^{c}	0.96 ^{c}	87.58 ^{c}	35.72 ^{c}	138.01 ^{c}
Pooled SEM	0.51	0.07	0.48	0.59	0.99
Sig.	**	**	**	**	**

a, b, c, Means in the same column with different superscripts, differ significantly ($p<0.05$). Sig.= Significance, ** ($p<0.01$). NS = Not Significant. SEM = standard error mean.

Table (7): Some physical semen traits of Bandarah cocks fed diets supplemented with different levels of propolis.

Parameters	Propolis levels (mg/kg diet)				Pooled SEM	Sig.
	0	150	300	450		
Ejaculate volume(ml)	0.57	0.58	0.59	0.60	0.15	NS
Sperm concentration(10^9/ml)	2.01 ^{c}	2.52 ^{b}	2.99 ^{a}	3.00 ^{a}	0.07	**
Sperm abnormality (%)	13.15 ^{a}	11.84 ^{b}	9.84 ^{c}	9.88 ^{c}	0.24	**
Sperm motility (%)	83.89 ^{c}	89.33 ^{b}	93.67 ^{a}	93.67 ^{a}	0.73	**
Sperm motility index	91.94 ^{c}	94.67 ^{b}	96.83 ^{a}	96.83 ^{a}	0.36	**

a, b, c, Means in the same row with different superscripts, differ significantly ($p<0.05$). Sig.= Significance, ** ($p<0.01$). NS = Not Significant. SEM = standard error mean.

Table (8): Fertility, hatchability % and chick weight at hatch of Bandarah laying hens fed diets supplemented with different levels of propolis.

Parameters	Propolis levels (mg/kg diet)				Pooled SEM	Sig.
	0	150	300	450		
Fertility %	86.84^c	94.06^b	96.72^{ab}	97.8^a	2.3	**
Hatchability of set eggs %	74.92^c	79.16^b	84.8^a	87.04^a	2.44	**
Hatchability of fertile eggs %	84.06^c	90.52^b	94.1^a	94.56^a	1.96	**
Chick weight (g) at hatch	35.4^d	37.9^c	41.33^b	43.6^a	1.14	**

a, b, c, d, Means in the same row with different superscripts, differ significantly ($p<0.05$). Sig.= Significance, ** ($p<0.01$). NS = Not Significant. SEM = standard error mean.

Table (9): Economic efficiency of Bandarah laying hens fed diets supplemented with different levels of propolis.

Items	Propolis levels (mg/kg diet)			
	0	150	300	450
Total feed consumption/hen(kg)	9.93	9.98	10.02	10.05
Price of propolis(L.E)	0.000	1.50	3.01	4.52
Total feed cost/hen (L.E)	34.76	34.93	35.07	35.18
Total cost(propolis+feed)	34.76	36.43	38.08	39.70
Total egg production /hen	37.15	41.66	49.42	51.27
Total price of egg (L.E)	55.73	62.49	74.13	76.91
Net revenue /hen	20.97	26.06	36.05	37.21
Economic efficiency (EE)	0.603	0.715	0.947	0.937
Relative (REE)	100.00	118.57	157.05	155.39

Feed cost/kg = 3.50 (LE), Price of one egg (fertile egg) = 1.50 (LE). Price of one gram of propolis= 1 LE. Net revenue = Total price of egg – Total cost.
EE = (Net return/total feed cost). REE, assuming control treatment = 100.

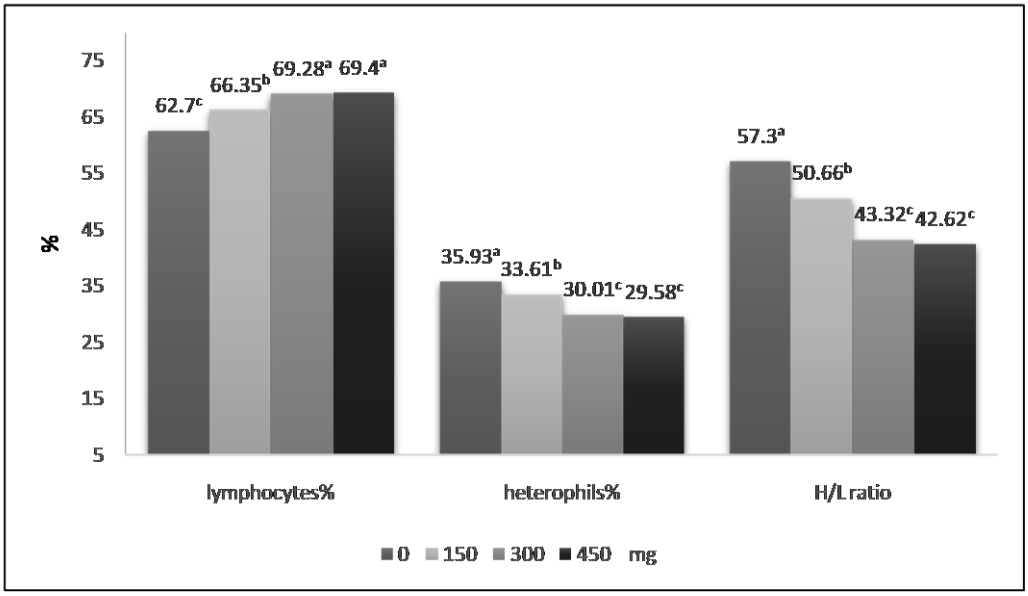

Fig. (1): Lymphocytes, heterophils and H/L ratio of Bandarah laying hens fed different levels of propolis.

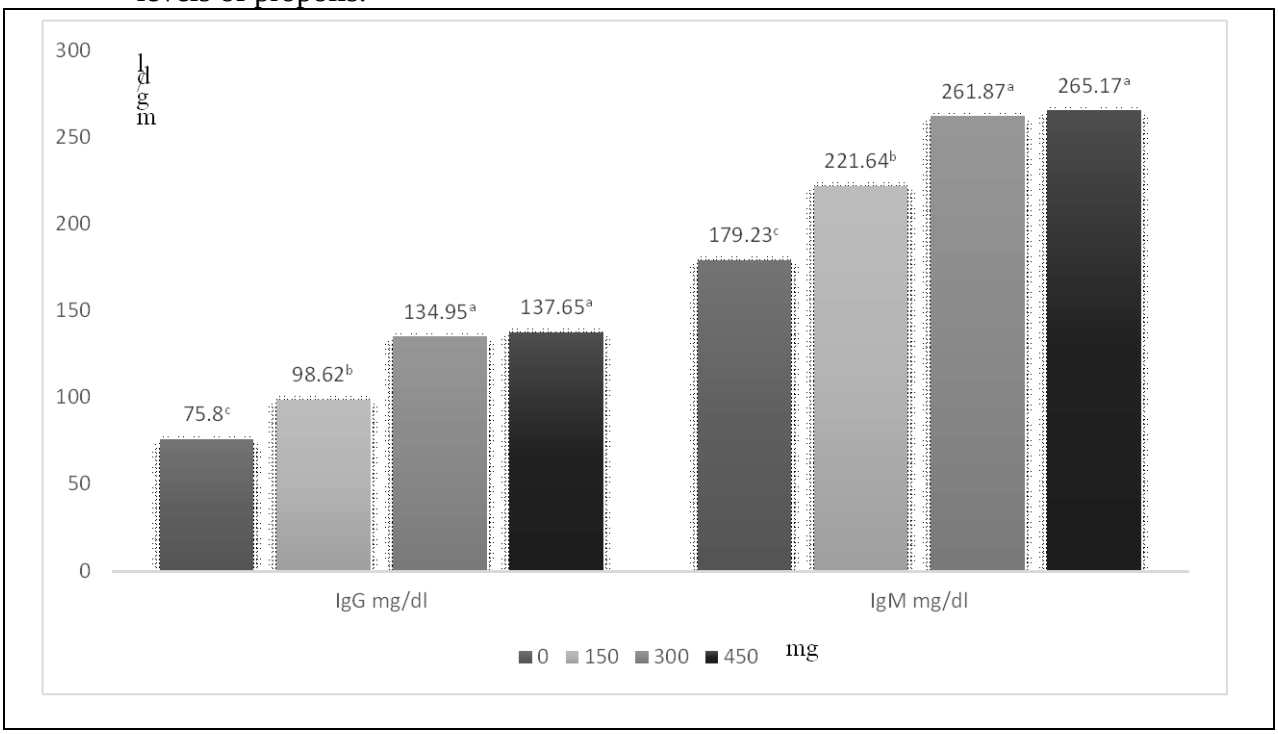

Fig. (2): Plasma IgG and IgM of Bandarah laying hens fed different levels of propolis.

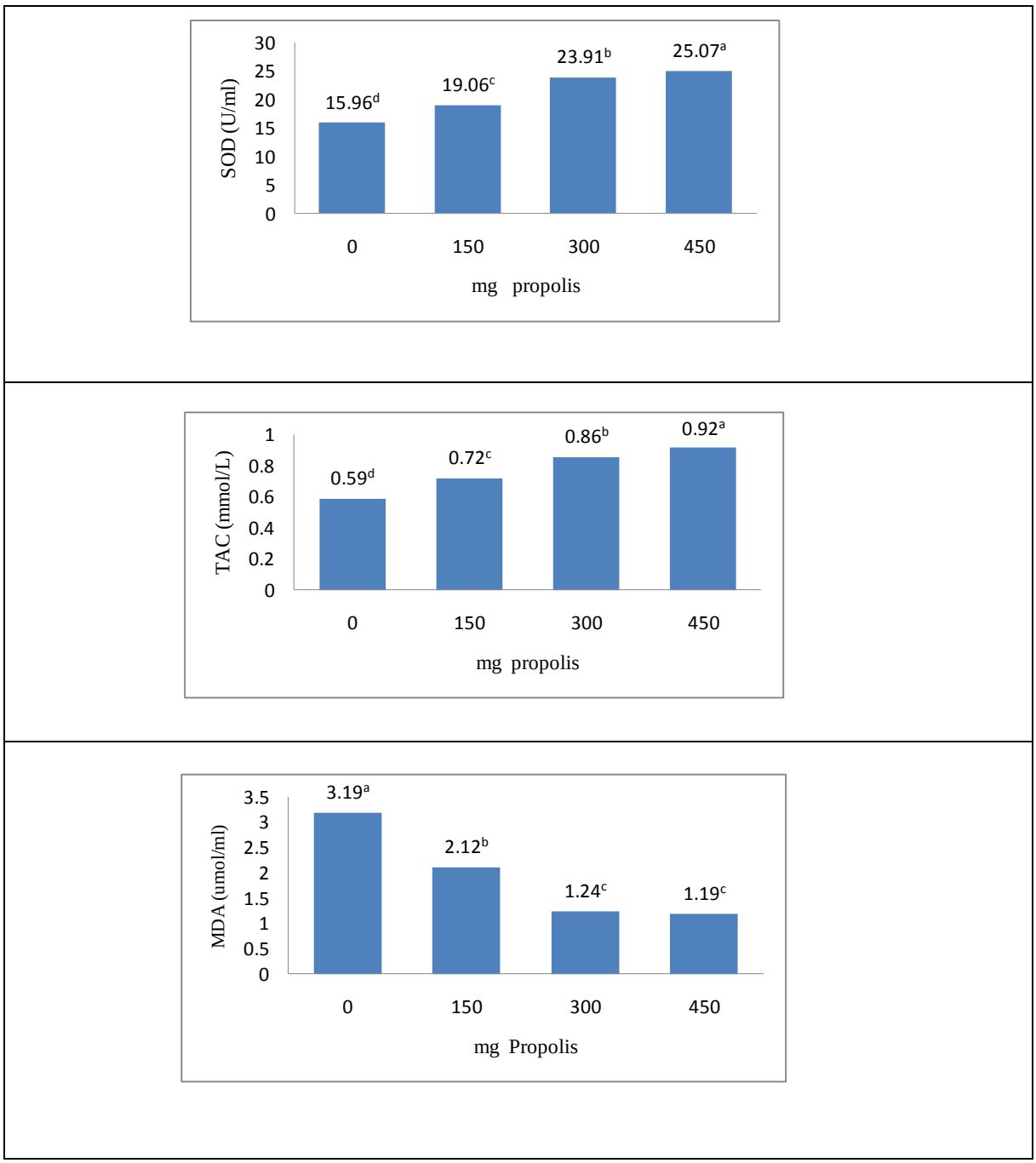

Fig. (3): Total antioxidants capacity (TAC), superoxide dismutase (SOD) and malondialdehyde (MDA) of Bandarah laying hens fed different levels of propolis.

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الملخص العربى

تأثير اضافة البروبوليس على أداء دجاج البندرة البياض

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تهدف هذه الدراسة الى تقيم تأثير اضافة البروبوليس الى العلف على اداء دجاجات وديوك البندرة. استخدم فى هذه الدراسة ١٢٠ دجاجة بندرة و ٣٢ ديك عمر ٣٠ اسبوع ، تم توزيعها عشوائيا الى اربع مجموعات كل مجموعة بها ثلاث مكررات بكل مكررة ١٠دجاجة + ١ ديك فى بيوت أرضية (٢,٥ x٢,٥١,٥م). استخدمت دجاجات المجموعة الاولى كمجموعة مقارنة (كنترول) وغذيت على العليقة الأساسية بدون اضافة والثلاث مجموعات التالية غذيت على العليقة الأساسية مضاف اليها البروبوليس بمعدل١٥٠ ٣٠٠ و ٤٥٠ ملجم /كجم علف واستمرت التجربة حتى عمر ٤٢ اسبوع.

اوضحت النتائج ان اضافة البروبوليس فى علف الدجاج البياض ادى الى زيادة معنوية فى انتاج ووزن وكتلة البيض و لم تؤثر الاضافات على معدل استهلاك الغذاء ولكن حدث تحسن معنوى فى نسبة التحويل الغذائى فى المجموعات المعاملة مقارنة بالكنترول. لوحظ تحسن معنويا فى جودة البيض نتيجة اضافة البروبوليس حيث ادى الى زيادة فى سمك القشرة و نسبة الصفار ووحدات هيو. لم تتأثر النسبة المئوية للقشرة و دليل شكل البيضة و دليل الصفار والنسبة المئوية للألبيومين و دليل الصفار وذلك بأضافة البروبوليس. أوضحت النتائج زيادة معنوية فى نسبة الهيموجلوبين والهيماتوكريت وعدد كرات الدم الحمراء والبيضاء فى المجاميع المعاملة مقارنة بالكنترول بينما انخفض معنويا عدد خلايا الهيتيروفيليس. لوحظ تحسن معنوى فى تركيز البروتين الكلى و الجلوبيولين وفى الحالة المناعية للدجاجات البياضة حيث زادت معنويا جلوبيولينات المناعة (IgM and IgG) بزيادة البروبوليس. وجد انخفاض فى الدهون الكلية والكوليستيرول المنخفض الكثافة وMDA وتحسن فى كفاءة الكبد والكلى فى المجاميع المعاملة مقارنة بالكنترول. اظهرت النتائج زيادة معنوية فى انزيمات مضادات الاكسدة (TAC, SOD) و انخفاض (MDA) فى المعاملات المضاف اليها البروبوليس. كما اوضحت النتائج تحسن فى جودة السائل المنوى ونسبة الخصوبة والفقس نتيجة اضافة البروبوليس حيث اظهرت المجاميع المعاملة تحسن فى الكفاءة الاقتصادية مقارنة بالكنترول.

ونستخلص من نتائج هذه الدراسة ان اضافة البروبوليس الى عليقة دجاجات وديوك البندرة ادى الى تحسن الاداء الانتاجى والتناسلى وجودة السائل المنوى والحالة المناعية وكانت افضل النتائج مع اضافة ٣٠٠ و٤٥٠ ملجم بروبوليس ولكن المستوى ٣٠٠ حقق اعلى كفاءة اقتصادية.