Egyptian Poultry Science Journal

http://www.epsaegypt.com

ISSN: 1110-5623 (Print) – 2090-0570 (On line)

EFFECT OF ADDING BEE BREAD AND BEE POLLEN AS ANTIOXIDANTS ON PRODUCTIVE PERFORMANCE AND FUNCYIONAL PROPERTIES OF HY-LINE HENS STRAIN

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Received: 29/08/2016 Accepted: 30/10/2016

ABSTRACT: The objective of this study was to investigate the effect of dietary bee bread (B), bee pollen (P) and their mixtures (BP) as antioxidants on productive performance and functional properties of table eggs in Hy-Line hens strain. A total number of 1000 Hy-line (W36) hens (27-wks-old) were weighed and randomly distributed into ten experimental groups, 100 birds each; five replicates (20 hens). The experimental design consisted of ten experimental groups as follow, the basal diet without B or P, the second diet was contained 0, 0.015 % butylated hydroxytoluene (BHT) (positive control) and other dietary treatments divided into eight groups include graded levels of B (0.1, 0.15, 0.2 %), P (0.1, 0.15, 0.2 %) and combination between B+P (0.05+0.05% and 0.1+0.1%). Results indicated that all the experimental diets produced greater egg number than control diet, but a clear improvement was observed in the hens fed diet contained 2g P/Kg diet followed by the diets supplemented with 0.15% P and 0.1%B+0.1%P. Egg mass/hen was significantly improved by the diet contained 2 g P /kg diet comparing with the control diet. The diets supplemented with 0.2% B, 0.15% P and 0.1% B recorded significantly lower values of triglycerides in fresh eggs yolk than control diet. The total cholesterol in stored yolks ranged from18.02 to 45.99 mg/ dl in the hens fed diets with BHT, 0.2% P, 0.1% B + 0.1% P, 0.15% B, 0.15% P and 0.2% B respectively while it was 63.79 mg/dl in control diet. The digestibility coefficient of ether extracts was significantly improved by the diet with 0.15% B and 0.15% P compared to the control diet. The diet contained 0.05% B+0.5% P, 0.2% B, 0.15% P and 0.2% P resulted in significantly higher serum total antioxidant records than control diet. Indeed. 0.2% B, 0.15% B, 0.1% P and 0.1% B in HY-Line hen's diets can be used as a antioxidant to enhance the production performance and internal egg yolk quality which are essential demand for consumer healthy "organic products".

Key Words: Bee bread, Bee pollen, Laying performance, functional properties, eggs

INTRODUCTION

Phytobiotics are a new group of natural products; they are defined as products derived from plants, which may have a beneficial effect on performance and quality of animal products. Numerous studies demonstrate that a great number of medicinal and aromatic herbs, as well as fruits and leaves of some berry plants biosynthesize phytochemicals possessing antioxidant activity and may be used as a natural source of free radical scavenging compounds (Sacchetti et al., 2005; Yu et al., 2005).

A free radical (R•) is a molecule or molecular fragment contains one or more unpaired electrons in its outer orbital. Free radical is conventionally represented by a superscript dot. Free radicals usually derived from metabolic processes and some dietary components. Dietary oxidized fatty acids are absorbed by the intestine, which then, initiate lipid peroxidation in the tissues (Lobo et al., 2010). Because of their extreme reactivity, they are included in the group of reactive oxygen species(ROS) . Important characteristics of the ROS are: 1- Extreme reactivity. 2- Short life –span. 3- Generation of new ROS by chain reaction. 4 -Damage various tissues. Free radicals join readily with other compounds, thus free radicals can affect dramatic changes in the body, and they can cause a lot of damage for example, peroxidation of PUFA (polyunsaturated fatty acids) in plasma membrane leads to loss of membrane functions. In addition, the DNA damage may directly cause inhibition of protein and enzyme synthesis and indirectly cause cell death or mutation and carcinogensis (Vasudevan and Sreekumari, 2001).

Insufficient amounts of antioxidants in the feed deficiency may increase the incidence of diseases and toxicoses (Young and Woodside, 2001). However, synthetic antioxidants are not always beneficial for human health (Lobo et al., 2010).

Bee pollen is a natural product, which is collected from plants by honey bees. Bee pollen is a rich source of indispensable amino acids, water and fat soluble vitamins, and flavonoids (Attia et al., 2011b, c).In the hive, bees do not consume either nectar or pollen directly; in both cases they induce biochemical processes, so nectar is transformed into honey and pollen into bee-bread (Krell, 1996). Bee bread is made of pollen, which has been gathered by bees and mixed with its digestive enzymes, carried back to the hive, packed into pellets and preserved with tiny bit of honey and bee wax. Bee bread is composed of well balanced proteins containing all essential amino acids, the full spectrum of vitamins(C, B1, B2, E, H, P, nicotinic acid, folic acid), pantothenic acid, pigments and other biologically active compounds, like enzymes as saccharase, amylase, phosphatases, flavanoids, carotenoids, hormones (Nagai et al., 2005). According to the study by Aliyazicioglu et al. (2005), bee bread is a product with high potential for use as a food supplement. Bee pollen is a product composed of bee's nutritionally valuable components, comprising a considerable amount of polyphenol components which act as effective antioxidant.

The positive effect of dietary bee bread and bee pollen can be expressed through better appetite, improved feed conversion, stimulation of the immune system and increased vitality (Perić et al., 2009). Furthermore, bee pollen reduce the risk of atherosclerosis and high antioxidant activity of these products related to the flavonoid content, protect internal organs (liver, kidneys) against damage (mainly by toxic agents), stimulate regeneration of damaged tissues, and demonstrate an anti-carcinogenic effect (Šarić et al., 2009).

The objective of this study was to investigate the effect of dietary bee bread (B), bee pollen (P) and their mixtures (BP) as antioxidants on productive performance and functional properties of table eggs in Hy-Line hens strain.

MATERIALS AND METHODS

Antioxidants sources:

The fresh bee bread and bee pollen was collected by beekeeper from the delta of Nile during 2015 and was kept at -5^{o}c for future use in the current study. Samples of bee bread and bee pollen were taken to determine approximate analysis which included crude protein, lipids and ash according to AOAC (1998) method.

Bioactive composition and antioxidant activity of bee-bread and bee pollen:

, Total phenols, flavonoids (mg/g) and the antioxidant activity by means of DPPH(2,2 diphenyl-1-picryl-hydrazyl) free radical scavenger capacity were determined in the laboratory of Food Technology Res. Institute, Agriculture Res. center according to the method by Ivanova et al., (2005) and Su and Silva (2006).

Experimental bird's procedure and management:

The current study was carried out at the integrated complex of egg production, National service projects organization, Ministry of Defense, Egypt. The experiment was conducted from 18August to 23 December 2015. One thousand Hy-line (W36) hens (27-wks-old) were weighed and randomly distributed into ten experimental groups, 100 birds each; five replicates (20 hens). The experimental design consisted of ten experimental groups as follow, the basal diet without B or P, the second diet was contained 0, 0.015 % butylated hydroxytoluene (BHT) (positive control) and other dietary treatments divided into eight groups include graded levels of B (0.1, 0.15, 0.2 %), P (0.1, 0.15, 0.2 %) and combination between B+P (0.05+0.05% and 0.1+0.1%). All birds were fed by 100g feed /day according to the recommendations of this strain, also it were reared under similar hygienic and managerial conditions. Throughout the experimental period, feed and fresh water were available all the time. The composition and calculated analysis of diet treatments are shown in Table 1.

Laying performance traits

Body weights of hens were recorded during the experiment period (27– 42 -wk-of age). Egg number and egg mass were recorded then were averaged and expressed per hen / four wk through the four periods (27-30, 31-34, 35-38 and 39-44 wks of age) and the overall experimental period (27-44 -wk- old). Laying rate and feed conversion ratio were calculated through the same periods as well as change body weight was calculated through the whole experimental period.

Chemical analysis of egg yolk

At 12 weeks of the experimental period, 4 eggs per replicate were collected. Eggs were divided into two portions of 10 eggs each. The 1st group was used to calculate fresh eggs quality, while the remaining 10 eggs were stored in the refrigerator at 5°C for 21 days and then broken for quality assessment. Yolk cholesterol was also determined after lipid extraction with a mixture of chloroform: methanol (2:1 v/v) using the procedure described by Folch et al. (1957). Egg cholesterol was determined according to the method of Richmond (1973), triglycerides according to AOAC (2004) methods and total antioxidant capacity by colorimetric method according to Koracevic et al., (2001).

Metabolism traits

At 42weeks of age 30 ♀ were selected on the basis of the average body weight (3 ♀ per treatment). Birds were individually housed in metabolic cages (60 cm long. 50 cm wide. 60 cm high) and fed their respective experimental diets (Table1), for a period of two days to allow the birds to become adapted to cages. Then, the excreta

were quantitatively collected every 24 hours for three days; feed consumption data were also recorded.

Any feathers or foreign debris were removed out. Then, the excreta were dried in a forced oven at 70 ^{o}C for 48 hours. Finally, the excreta were ground well and stored in plastic bags in pledge of analysis.

The proximate analysis of experimental diet and the excereta were carried out according to the official methods (A.O.A.C., 1998). The procedure described by Jakobsen et al., (1960) was used for separating fecal protein in excreta samples.

Digestion coefficients for protein , organic mater and crude fiber were calculated according to the following equation:

Digestion coefficient% = [(Nutrient intake (g) – Fecal nutrient content (g)) / Nutrient intake (g)]. 100.

Hematology analysis of blood:

Blood samples were collected and used to determine the total leukocytes (WBC) according to Natt and Herrick (1952). Blood smears were made and stained for differential leukocyte counts (100 cells/ smear, Cook, 1959) and means were calculated for heterophils, lymphocytes, monocytes, basophils and heterophils: lymphocytes (H/L) ratio.

Biochemical analysis of blood serum:

At the end of the experimental period, 1 hens from each replicate (5 hens/ treatment) were chosen randomly, blood samples were collected and divided into two halves. The blood sample was centrifuged at 3500 rpm for 15 minutes , to separate the serum for biochemical analysis, which include ALT, AST, total protein and albumin by using commercial kits. The globulin concentration was obtained by subtracting the values of albumin from the corresponding value of total protein.

Also, total antioxidant was determined in serum by commercial kit as indicator of oxidation stress in living system according to Koracevic et al., (2001).

3.10. Economic efficiency and net return:

Data were calculated based on the prices of bee bread (50 LE/ one kg) , bee pollen (100 LE/kg) , 150 g BHT 100% (15 LE) and one kg of table egg (0.65 LE) prevailing during year 2015. Economical efficiency for egg production was expressed as hen-production and calculated using the following equation:

Economic efficiency (%) = (Net return LE/Total feed cost LE) × 100.

Statistical analysis:

Data were analyzed by the analysis of variance according to SPSS (2008) and significant differences among means were detected by the Duncan's Multiple Range Test (Duncan, 1955).The following model was used : $Yij = \mu + Ti + eiJ$ where, Yij = an observation, μ = overall mean, Ti = Effect of treatment (1, 2 , … , 19) and eiJ = Random error

RESULTS AND DISCUSSION

Nutritional, bioactive composition and antioxidant activity of bee-bread and bee pollen:

As shown in Table (1), the chemical analysis of B and P is noticeable where moisture, crude protein, ether extract and ash of bee bread were nearly similar in both B and P except for the crude protein where it was higher in B than P by about 5.1%.. In addition, the total phenols, total flavonoids and antioxidant activity by DPPH radical % in B were 22.83 mg/g, 6.77 mg/g and 97.4 % in respectively. While in P, the total phenols, total flavonoids and antioxidant activity by DPPH radical % were 14.2, 5.65 mg/g and 97.33 % respectively.

The results in the current study consist with the study by carpes et al., (2009) who found that the contents of phenolic compounds and total flavonoids were 30.46 ± 8.22 mg of gae.g–1 pollen and 8.92 ± 5.5 mg of quercetin.g–1 pollen, respectively.

also, nijveldt et al., (2001) found that total phenolic content in bee-bread ranged from 2.5 to 13.7 mq eq-gallic acid / g bee-bread.

in respect of the antioxidant activity, such properties are related to chemical structure, especially, total phenolic, flavonoids, vitamins, minerals and other natural antioxidants so that the results illustrated that the antioxidant activity of bee bread (97.4 %) was slightly higher than the value reported by the b (97.33 %). according to the study by nagai et al., (2005) b is made of pollen that has been gathered by bees and mixed with its own digestive enzymes. for that reason, bee bread in itself may be possess an antioxidant activity and scavenging activities against active oxygen species. also, in study by zuluaga et al., (2015), antioxidant activity reported values ranged from 46.1 to 76.3 μmol trolox/ g b, in comparison to reported values for bee-pollen, 76.2 μmol trolox/ g p. the antioxidant activity of polyphenols is due to their redox properties, which play an important role in scavenging free radicals and oxygen species or decomposing peroxides; such properties are closely related to its chemical structure, especially with the number of hydroxyl groups on the aromatic ring and conjugated double bonds. in addition to their individual effects, antioxidant molecules interact synergistically so that one can protect other against oxidative destruction (damintoti et al., 2005).

Live body weight

The results in Table (3) clearly observed that the hens fed diet supplemented with 2 g P/Kg diet had significantly lower ($P \leq 0.05$) final body weight (FBW) than control diet at 42 weeks of age. On the other hand, the FBW of hens given the low level of B (0.1%) exceeded those of hens fed diet without any supplementation, however all treatments with exception the diet with 0.2% P did not significantly ($P \geq 0.05$) differ from the control diet as their FBW where it was more or less equal to 2.1 to 1.36 % of the control record.

In respect of body weight gain (BWG), the results revealed that the hens fed the diet supplemented with 2g P/Kg diet had lower BWG records as comparison with the control diet by about 23.54%. Similar results were reported by Ali et al., (2007) who showed that the addition of thyme as a natural antioxidant at level 0.25% significantly decreased weight gain in layer hens compared of hens fed control. But, , there is somewhat discrepancy between the results in the current study and the results of **Awad et al., (2013)** who reported that no significant differences in LBW and BWG in layers hens due to supplementing bee bread as a natural antioxidant at levels of 0 , 0.5 , 1.0 and 1.5 g / kg diet.

This decrease in BWG as a result of supplemented the layer diet with 2g P/Kg may be due to the phenolic compounds in the P where the results by Kang et al., (2015) indicate that polyphenol and flavonoids augments the anti-obesity activity by regulating the expression of the genes involved in lipid accumulation. Physiologically any increase above this average record indicates that hens tended to obesity and reflects the incidence of abdominal and visceral fat deposition, a matter which is considered a disadvantage especially with egg laying hens.

Zuluage et al., (2015) illustrates the potential mechanisms of dietary polyphenols on anti obesity. Many lines of research indicate that dietary polyphenols prevent obesity development through the following possible mechanisms:

(1) Lower food intake, (2) decrease lipogenesis, (3) increase lipolysis, (4) stimulate FA β-oxidation, (5) inhibit adipocyte differentiation and growth, and (6) attenuate inflammatory responses and suppress oxidative stress.

The productive performance:

Egg number and egg weight:

As shown in Table (4), the results of egg number / hen indicated that all dietary treatments did not actually differ from control diet in the period from 27- 30 weeks of age. The same manner, in comparison with the control diet no significant alternations were reported in egg number due to using any of the dietary treatments during the second period (31-34 wk of age), however hens fed the diet supplemented with 0.2% P produced higher egg number than control diet by about 4.07%.

On the other hand, the diets contained 0.15% and 0.2% P resulted in a significant increase ($P \leq 0.05$) in egg number compared to the control diet while, the other treatments did not significantly ($P \geq 0.05$) differ from control value during 35-38 wk of age. But, the most remarkable result during the third period is that the diet with 0.1% B and 0.05% B+0.05% P did not significantly ($P \geq 0.05$) differ from the diets supplemented with0.15% and 0.2%.

Results of the period from 39-42 weeks of age confirmed the superiority of diet with 0.02% B at egg number as it was significantly greater ($P \leq 0.05$) than control group. Also, the other treatments resulted in a significant improve ($P \leq 0.05$) in egg number/hen with exception the control positive and the diet with 1g P/Kg diet as compared to the control diet.

Dealing with the collective data of the whole four periods, it could be mentioned that all the experimental diets produced greater egg number than control group, but a clear improvement was observed in the hens fed diet supplemented with 0.2% P which gave significantly higher ($P \leq 0.05$) egg number than control by about 4.82% followed by the diets supplemented with 0.15% P and 0.05% B + 0.05% P.

In respect of egg weight, generally results obtained clearly indicated that no significant influence on egg weight except for the diet with 0.15% B and 0.2% B where it produced significantly lower ($P \leq 0.05$) egg weight than the control diet during the collective period (27-42 weeks of age).

Egg mass

AS shown in Table (5), during the collective period (27-45 weeks of age), egg mass / hen was significantly improved ($P \leq 0.05$) by the dietary with 0.2% P comparing with the control diet, while the improvement in egg mass du to the other treatments was insignificant ($P \geq 0.05$) compared to the control group. The results showed that the highest values of egg mass / hen were by hens fed diets contained 0.2% P, 0.1% P, 0.05% B + 0.05% P, 0.% B, 0.2% B and 0.1% B + 0.1% P ranging from 101.0 to 104.5 % of control value.

Feed conversion ratio

Feed conversion ratio during the experimental periods fluctuated from month to month and had no fixed trend (Table 5). In general, the overall means during the whole period showed that all treatments did not significant differences in feed conversion ratio compared to the control diet.

It is mentioned to that these results confirmed those reported by Attia et al. (2011 b, c) and Popiela-Pleban et al. (2012) who indicated that bee pollen could be utilized as natural growth promoters in poultry production. The positive influence of B or P on poultry performance is in line with those reported by several researchers for example (Popiela- Pleban et al., 2012). In addition these results in line with findings of Awad et al., (2013) who found that egg number per hen was significantly improved by feeding diets supplemented with bee bread levels at 32-35, 36-39, 40-43 and 24-47 wks of age as compared to the control group.

Pollen contains significant amounts of polyphenolic substances, mainly flavonoids (Almaida-Muradian 2005). Nowadays, the interest in phenolic compounds has increased due to the antioxidant and free

radical scavenging activities (Dorman et al., 2003). In addition, from nutritional value of the phenolic compounds Several experimental animal studies have suggested that the consumption of bioactive polyphenolic contents with potent antioxidant activities, may provide several health benefits including improvement in cognitive function (Joseph et al., 1999), antioxidant effects (Youdim et al., 2000), and modulation of obesity and adiposity.

The different results between bee bread and pee pollen may be due to the composition of bee bread differs slightly from that of bee pollen. It has higher acidity due to the presence of lactic acid and a large amount of vitamin K. The quantity of lactic acid is six times larger than bee pollen. The higher activity of bee bread causes a good preservation of bee bread due to the inhibition of the growing of molds as well as microorganisms (Nagai et al., 2005). According to Wang et al. (2007) reported that bee pollen might potentially work as a trophic agent to enhance small intestinal function in broiler chickens.

It is clear that, the antioxidant function is to act as a coordinated and balanced system to protect tissues and body fluids from damage by free radicals whether produced physiologically or as a response to inflammation infection or disease (Turrens et al., 1984). Moreover, Halliwell , (1996 b) reported that in health, the balance between free radicals and the antioxidant defenses lies slightly in favor of the reactive species so that they are able to fulfill their biological roles. Repair system takes care of damage which occurs at a low level even in healthy individuals. From the results of the current stud it could be suggested that the effect of the natural antioxidants seemed to act gradually and persisting for longer period.

For these reasons, it was not strange to find that all bee bread, bee pollen and mixtures of them supplementation showed better egg production performance than control diet. It is remarkable that when the low level of B and P was supplemented together (0.05g B+0.5 P/Kg diet), the egg production % increased being about 3.2 % from control while the improvement resulted from the diet supplemented with 1g B or 1g P /Kg diet was 2.73 and 0.24% respectively compared to the control diet suggesting a synergistic effect.

The chemical composition of fresh yolks

Results in Table (6) observed the effect of the experiment different levels of B, P and BP adding on the chemical composition of fresh eggs yolk at 38 weeks of age. It showed that the diets contained 0.2% B, 0.15% P and 0.1% B recorded significantly ($P \leq 0.05$) lower values of triglycerides in eggs yolk than control diet, while the other dietary treatments had no significant influence on the triglycerides in eggs yolk. In respect of total cholesterol in yolk, all treatments resulted in a decrease in the total cholesterol in yolk, but this effect was significantly du to the diets contained 0.1% B, 0.15% B, 0.2% P and 0.1% B+0.1% P compared to the control diet.

In addition, all treatments improved the total antioxidant values in eggs yolk, where hens fed the control diet recorded the lowest value of total antioxidant than all the experimental groups except for the diet included 0.1% B.

The chemical composition of stored yolks:

Results concerning the chemical composition of stored eggs yolk from Hy-line laying hens for 21 days at 5°c at 38 weeks of age as influenced by the dietary treatments are given in Table (6).

Comparison with the control diet, triglycerides, cholesterol and total antioxidant in stored yolks for 21 days were had no significantly ($P \geq 0.05$) affected by the dietary supplements applied. However, it is remarkable is that the best values of triglycerides in stored yolks were those of the diets with 0.1% B and 0.05%

B+0.05%P being significantly lower than the diets supplemented with 0.15% B, 0.2% B, 0.15% B and 0.2% B.

Indeed, the most remarkable result is that all treatments except the diet contained 0.2% resulted in decrease the total cholesterol in stored eggs yolk by about 20.22 (0.2% P) to 61.25 (0.1% P) as compared to the control diet, this refer to the importance of B and P as natural antioxidant in the layer's diets where eggs are one of the most widely consumed animal food products and are generally considered to be an important source of unsaturated fats, essential amino acids, and B vitamins. There is also some evidence to suggest that consumption of eggs can decrease blood glycemic index (Pelletier et al., 1996) and raise high density lipoprotein cholesterol (HDL) levels compared to carbohydrate rich diets (Schnohr et al., 1994). A 20 ounce egg contains between 186-213 mg of cholesterol, and some studies have linked high dietary cholesterol to increased risk of cardiovascular disease (Krauss et al., 1996). The American Heart Association's Nutrition Committee still recommends limiting dietary cholesterol intake to 300 mg per day or 200 mg per day for people with heart disease or diabetes (Lichtenstein et al., 2006).

In respect of the total antioxidant in stored yolks, it is clear that dietary treatments did not any significant alternations compared to the control group.

The results in the current study illustrated that the bee products (B and P) resulted in improve the internal egg quality in terms of the triglycerides, cholesterol and total antioxidant especially in fresh eggs yolk, there is no discrepancy between our results and the findings by Miura et al. (2001) who reported that natural antioxidant reduces the content of cholesterol and triglycerides by up to 27 to 50%. In addition, Ali et al. (2007) found that the hens fed thyme (natural antioxidant) significantly recorded the lowest values of total cholesterol.

Similar to the present results, Abd El-Baky et al. (2008) suggested that natural antioxidant is a valuable source to increase shelf life of foodstuffs, rather than synthetic antioxidants such as BHT and BHA and can prevent cellular damage. The reduction of cholesterol and increase total antioxidant in yolks by fed diets supplemented with natural antioxidant may be explained as follow:

1- Natural antioxidant decreased oxidative profiles and cholesterol due to decreasing cholesterol absorption. 2- Raederstorf et al. (2003) and Botsoglou et al. (2005) found that lower value of malondialdehyde in eggs from the hens fed Saffron or "-tocopherol as compared to controls and they suggested that possible transfer of antioxidant constituents of saffron into the hen through feeding might inhibit the chain reaction involved in oxidation of the consumed lipids, thus decreasing the oxidation products transferred into yolk. 3- Panda et al. (2003) hypothesized that the cholesterol - lowering effect was due to reduced cholesterol absorption from the gastrointestinal tract and/or by the deconjugation of bile salts in the intestine, which would prevent their reabsorption via the enterohepatic circulation.

Digestibility coefficient of nutrients

The results in Table (7) showed that no significant ($P \geq 0.05$) influence of dietary treatments on digestibility coefficient of DM, CP, NFE and TDN % compared to the control diet. However, the digestibility coefficient of ether extract significantly improved ($P \leq 0.05$) as a result of feeding the diet contained 1.5 g B and 1.5g P/Kg diet compared to the control diet. Also, the digestibility coefficient of crude fiber significantly increased ($P \leq 0.05$) by about 46.86 and 38.34% du to the diets with 0.1% P and 0.1% B+0.1% P respectively comparing with the control diet. In addition, the hens fed diets with 0.5%

B+.05% P resulted in a significant (P≤0.05) improve in the digestibility coefficient of organic matter (OM) being about 1.42% compared to control diet.

These results in the line agree with the findings of Radwan et al. (2008) who reported that the addition of Vit. E (100 or 200 mg/Kg) or herbs (0.5 or 1.0%) to hen's diets numerically increased the nutrient digestibility coefficients.

The likely reasons for the improvement in the digestibility coefficient of EE, CF and OM results from fed diets supplemented with B or BP are speculative, this may be explained according to study by (Nagai et al., 2005) where who reported that the enzymatic hydrolysates were prepared from bee bread by digestion of three kinds of enzymes (pepsin, trypsin, and papain). Bee bread was successfully digested and the yields of these hydrolysates were as follows: 10% (pepsin hydrolysate), 10% (trypsin hydrolysate), and 4% (papain hydrolysate) on the dry weight basis, respectively. Zuluaga et al., (2015) illustrated that the digestibility parameter has shown significant differences between the found value for bee-bread (79.1 g protein digested/100 g total protein) and bee-pollen (63.9 g protein digested/100 g total protein). Despite being an in vitro test, it reflects the variation in the structure of pollen and the greater availability of nutritional and bioactive compounds in bee-bread.

The hematology traits

Statistical analysis revealed that all dietary treatments had no significant (P≥ 0.05) effect on content the blood of red blood cells (RBC) compared to the control diet (Table 9), however, the diet supplemented with 0.1% P, 0.15% P, 0.2% P, 0.05% B+.05% P and 0.1% B+0.1%P resulted in improve the RBC values by about range from 4.63 to 13.21 % comparing to control group. In addition red blood cells in hens fed diet with .02% P and 0.1% B + 0.1% P was significantly increased (p≤ 0.05) compared to the control positive.

White blood cells (WBC):

Regarding the total WBC's count, it could be seen that the hens received diet with 0.15% P, 0.1%B + 0.1% P and 0.2% P had significantly the highest value of WBC's count than control diet, but the other dietary treatments did not significantly differ from control diet.

Heterophils % (H %):

As shown in Table (9), dietary treatments did not appear to influence H%, however, it spite of the apparent seen among groups, these results may be to the great variation within groups, it could be observed that the least value of H % was to hens fed the diet supplemented with 0.2% P and the highest value was those fed diet with 0.1% B, while the other treatments were more or less equal.

Lymphocytes % (L %):

No significant influence of dietary B, P and BP on the percentage of Lymphocytes could be detected (Table 9). But, it was observed that the diets supplemented with the higher dose of 0.2% P and 0.05% B+0.05% caused a significant increase (P≤ 0.05) of L % compared to the diet with 0.1% B , suggesting some kind of synergistic between B and P.

H/L ratio:

It is evident that the H/L ratio was significantly decreased (P≤ 0.05) for hens fed 0.2% P and 0.05% B + 0.05%P compared to those fed 0.1% B , while, as compared to the control diet, all dietary treatments did not significantly (P≥0.05) differ from control group.

Monocytes and eosinophil's cells % (M and E %):

It was also noticed that the increase in M % caused by the diet supplemented with 0.1% P, 0.15% P and 0.05% B + 0.05%P was significantly (P≤0.05) higher than that caused by the control diet. Regarding the E %, all treatments groups did not show any

significant differences in this value compared to the control diet.

The results in the current study illustrated that the supplementation of B and P in the Hy- line hen's diet improved the immunity of hens during the period of egg production from 27-42 wks. of age where the hens exposure to metabolic stress especially through the peak of egg production, as free radicals derive from metabolic **processes** and some dietary components. Insufficient amounts of antioxidants in the feed, e.g. vitamin E (vitamin E) deficiency, may increase the incidence of diseases and toxicoses (Young and Woodside, 2001). Natural antioxidants, such as tocopherols, vitamin C, flavonoids and synthetic antioxidants, such as butylated hydroxytoluene (BHT) are generally used to slow down or stop lipid peroxidation and preserve product freshness. However, synthetic antioxidants are not always beneficial for human health (Lobo et al., 2010).

These results were supported by many of scientific publications, for example Attia et al., (2011b,c) reported that bee pollen can enhance immunity, promote growth, protect gut health, and improve quality and safety of animal products. Also, the study by Nagai et al., (2005) reveals that enzymatic hydro lysates from bee bread are of benefit not only for the materials of health food diets, but also for in patients undergoing various diseases such as cancer, cardiovascular diseases, diabetes, and hypertension. The apicultural products such as pollen and bee bread, has gained increased attention for its therapeutic properties, as immunomodulatory (Gebara et al., 2002), antibacterial (Proestos et al., 2005), antifungicidal (Garci et al., 2001), and anti-caryogenic (Almas et al., 2001) effects. Bee gathered pollen is considered a valuable special food with varied enhancing effects in health (Bogdanov, 2004). Several researchers (Galal et al., 2008; Attia et al., 2011b,c and Popiela-Plebanetal., 2012) demonstrated that bee pollen could be used as growth promoters and immune enhancers as an alternative to antibiotics.

Serum biochemical

The results in Table (9) showed that serum total protein was not significantly affected by the dietary treatments. However, all treatments were slightly higher than control diet except for the diet supplementation wit 0.05% B + 0.05% P where it recorded the lower value of total protein than control and other groups.

The same manner, serum albumin content was not significantly ($P \geq 0.05$) caused by all the dietary treatments comparing with control diet, but the serum hen's fed diet with 0.15% B recorded higher serum albumin than the diet contained 0.1% B.

Also, no significant ($P \geq 0.05$) alternations were recorded in serum globulin content due to using any of dietary treatments. But, it is clear that the most treatments improved the value of serum globulin content especially the hens fed diet supplemented with 0.2% B compared to the control diet.

The positive effect of bee pollen on serum metabolites could be attributed to its contents of vitamins, minerals, and phospholipids (Leja etal., 2007) and antioxidant effects (Quian etal.,2008; Šaric etal.,2009). In addition its high contents of macro and micronutrients, among which amino acids, polyunsaturated fatty acids, and minerals (Leja etal.,2007; Šaric etal., 2009) can be easily absorbed as bee pollen constituents in mice has been shown top as s from the stomach into the blood stream within 2 h after ingestion (Markham et al.,1996).

Serum activity of AST and ALT

Results obtained clearly observed that all dietary treatments had no significant ($P \geq 0.05$) effect on serum activity of AST compared to the control diet. On the other hand, the results indicated that serum ALT was significantly increased ($P \leq 0.05$) when

the hens fed control positive, 0.2% B and 0.15% P compared to the control diet. In addition, all treatments resulted in insignificant increased (P≥ 0.05) in serum ALT content comparing with the control group

The effect of B and P on plasma metabolites could be attributable to its contents of vitamins, minerals, and phospholipids (Leja et al.,2007) and antioxidant effects (Quian et al.,2008; Šaric et al.,2009). In addition, the decrease in plasma cholesterol could be due to phospholipids and polyunsaturated fatty acids, particularly linolenic fatty acid in bee pollen (Xu et al.,2009).

Serum antioxidant activity

Results of serum antioxidant activity come not strange where the hens fed diet contained 0.05% B + 0.05% P, 0.2% B, 0.15% P and 0.2% P /Kg diet showed significantly higher records than control diet by about 47.33, 39.7, 38.17 and 37.4% respectively. Meanwhile, the other dietary treatments had no significant effect on total antioxidant activity compared to the control diet.

It is remarkable that when low levels of B and P were added together (1g BP/Kg diet) the serum antioxidant activity increased significantly (P≤0.05) compared to control and the low level of B and p alone suggesting an synergistic effect. Our results indicated that total antioxidant activity was significantly higher in serum of hens receiving the combination of B+P.

Regarding the antioxidant activity due to supplementation of B and P, the antioxidant action has been attributed to several phenolic compounds with antioxidant activity, Caldwell, (2003) found that polyphenols are antioxidants with redox properties which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers.

The economic efficiency (EE):

The data obtained in Table (9) represents the economic efficiency (EE) of egg production in response to the dietary supplements investigated.

The results in the present study illustrated that no significant in EE of egg production could be detected among the experimental groups and the control diet, however, the results clearly observed that the hens fed diets contained 0.1% B, 0.05% B+ 0.05%P and 0.2% B produced the highest values of relative EE of egg production as compared to the control diet.

CONCLUSION

According to the current results obtained the Hy-line hens fed diets contained 0.2% B, 0.15% B, 0.1% P and 0.1% B as a antioxidant can be used to enhance hen's welfare, production performance and internal egg quality in terms of triglycerides, cholesterol and total antioxidant in eggs yolk which are essential demand for consumer healthy "organic products".

Table (1): Bioactive compounds and antioxidant activity of bee bread and bee pollen

Chemical analysis	feed adding	
	Bee bread	**Bee pollen**
Moisture (M) %	10.3	15.16
Crude protein(CP) %	24.2	19.1
Ether extract (EE)%	2.85	2.43
Ash %	3	3.05
Crude fiber (CF) %	3.4	3.4
NFE %[1]	56.25	59.91
ME (Kcal/Kg)[2]	2229	2080
Total phenols (mg/g)	22.83	14.2
Total flavonoids (mg/g)	6.77	5.65
Antioxidant activity by DPPH radical %	97.4	97.33

[1]NFE= 100- (M+ CP+ EE+ ash+ CF) [2]Metabolizable energy (ME, kcal/kg) was calculated according to NRC (1994). ME = 36.63 × CP + 77.97 × EE +19.87 × NFE.

Table (2): Composition and calculated analysis of the experimental diets

Diets Ingredients (%)	C	The experimental diets								
		PC	0.1B	0.15B	0.2B	0.1P	0.15P	0.2P	0.05B+ 0.05P	0.1B+ 0.1P
Yellow corn	63.16	63.15	63.06	63.01	63.06	63.06	63.01	63.06	63.06	63.06
Soy bean meal (44 %)	14.54	14.54	14.54	14.54	14.44	14.54	14.54	14.44	14.54	14.44
Corn gluten (60 %)	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
Soybean oil	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Bee breed	-	-	0.10	0.15	0.20	-	-	-	-	-
Bee pollen	-	-	-	-	-	0.10	0.15	0.20	-	-
Bee breed + bee pollen	-	-	-	-	-	-		-	0.05 + 0.05	0.1 + 0.1
Di-calcium phosphate	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1
Limestone	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5
Vit & Min. premix[1]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
NaCl	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
DL- Methionine (99%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Lysine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Antiposition	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
BHT[3]	-	0.015	-	-	-	-				
Total	100	100	100	100	100	100	100	100	100	100
Calculated Analysis[2]										
Crude protein %	16.09	16.09	16.10	16.11	16.10	16.10	16.11	16.10	16.10	16.10
ME (Kcal / kg)	2834	2833	2836	2837	2839	2836	2837	2837	2836	2837
Crude fiber %	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Ether extract %	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2
Calcium (%)	4.49	4.94	4.49	4.49	4.49	4.49	4.49	4.49	4.49	4.49
Av. Phosphorus (%)	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.51
Methionine %	0.45	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46
Lysine	0.84	0.84	0.84	0.83	0.83	0.84	0.84	0.84	0.84	0.83
Price [4]	3.19	3.23	3.22	3.24	3.25	3.27	3.31	3.35	3.25	3.3

[1]Each 3kg of Vit .and Min. premix contains 100 million IU Vit. A;2 million IU Vit.D_3;10 g Vit.E; 1 g Vit.K_3 ; 1 g Vit B_1; 5 g Vit B_2 ;10 mg Vit.B_{12} ; 1.5 g Vit. B_6; 30 g Niacin ;10 g Pantothenic acid ;1g Folic acid;50 mg Biotin ; 300 g Choline chloride; 50 g Zinc; 4 g Copper; 0.3 g Iodine ; 30 g Iron; 0.1 g Selenium; 60g Manganese ;0.1 g Cobalt; and carrier CaCO3 to 3000 g .

[2] According to NRC 1994

[3] BHT= butylated hydroxytoluene

[4] Price of one kg (LE) at time of experiment for different ingredients : yellow corn, 2.27; Soy been meal, 5.05; Corn gluten, 6.50; Wheat bran, 2.22; 0.80; Di-calcium, 4.55; limestone, 1.50; Vit. & Min., 20.0; Na cl, 0.50 ; Meth, 32.0; Lysine, 32.0; Bee bread 50 and bee pollen 80.0

Table (3): Effect of adding bee bread, bee pollen and their mixtures on Change body weight of HY-Line laying hens

Treatments	Body weight (BW- g/hen)		
	Initial BW	FBW	BWG
[1]C	1426.8	1584.8^{abc}	158.0^{ab}
[2]PC	1434.6	1574.8^{abc}	140.2^{ab}
[3]$B_{0.1}$	1415.4	1617.8^{a}	202.4^{a}
[4]$B_{0.15}$	1400.8	1558.2^{cd}	157.4^{ab}
[5]$B_{0.2}$	1428.0	1576.8^{abc}	148.8^{ab}
[6]$P_{0.1}$	1414.4	1569.0^{bc}	154.6^{ab}
[7]$P_{0.15}$	1402.8	1562.4^{cd}	159.6^{ab}
[8]$P_{0.2}$	1404.0	1524.8^{d}	120.8^{b}
[9]$BP_{0.05+0.05}$	1416.4	1591.0^{abc}	174.6^{ab}
[10]$BP_{0.1+0.1}$	1448.2	1607.2^{ab}	147.0^{ab}
±SE	4.66	5.25	6.42
Sig.	NS	0.05	0.05

[1]C=control diet, [2]PC= control positive, [3]$B_{0.1}$=bee bread 0.1% in the diet, [4]$B_{0.15}$, [5]$B_{0.2}$=bee bread 0.15% in the diet, [5]$B_{0.2}$= bee bread 0.2% in the diet, [6]$P_{0.1}$= bee pollen 0.1% in the diet, [7]$P_{0.15}$= bee pollen 0.15% in the diet, [8]$P_{0.2}$= bee pollen 0.2% in the diet, [9]$BP_{0.05+0.05}$= bee bread .05%+bee pollen0.05% in the diet, [10]$BP_{0.1+0.1}$= bee bread 0.1+ bee pollen0.1% in the diet.

a,b,c :means in the same row bearing different superscripts are significantly different ($p \leq 0.05$). NS= Non significant

Table (4): Effect of adding of bee bread, bee pollen and their mixtures on egg number and egg weight of Hy-line laying hens during the period from 27 to 44 weeks of age

Tre. Age	C	PC	*B 0.1	B 0.15	B 0.2	*P 0.1	P 0.15	P 0.2	*BP 0.05+0.05	BP 0.1 + 0.1	±SE	Sig.
Egg number / hen / period												
27-30	27.2	27.4	27.9	27.8	26.8	27.0	27.0	27.3	27.3	27.3	0.16	NS
31-34	27. 0^{ab}	27.1^{ab}	27.1^{ab}	26.1^{b}	27.1^{ab}	27.2^{ab}	27.5^{ab}	28.1^{a}	27.4^{ab}	26.6^{b}	0.15	*
35-38	25.9^{cd}	26.4^{bcd}	26.4^{bcd}	26. 9 ^{abc}	26.3^{bcd}	25.7^{d}	27.2^{ab}	27.5^{a}	26.7^{abcd}	26.5^{bcd}	0.05	*
39-44	27.8d	29.0b^{cd}	29.5^{ab}	29.7^{ab}	30.5^{a}	28.1^{cd}	30.1^{ab}	30.0^{ab}	29.9^{ab}	29.1b^{c}	0.17	*
27-44	107.8b	109.8^{ab}	110.9^{ab}	110.5^{ab}	110.6^{ab}	108.1^{b}	111.8^{ab}	113.0^{a}	111.4^{ab}	109.5^{ab}	0.45	*
Egg weight (g/egg)												
27-30	56.2a	56.1a	55.1bc	54.7c	55.4^{abc}	55.8^{ab}	55.3^{abc}	55.6^{abc}	55.4^{abc}	55.6^{abc}	0.11	*
31-34	58.5a	58.0ab	57.9abc	57.1	57.3	58.1ab	58.5a	58.4a	58.1ab	58.3a	0.10	*
35-38	58.6^{a}	58.6^{a}	58.4^{ab}	58.1^{ab}	57.9^{b}	58.3^{ab}	58.5^{a}	58.4^{ab}	58.6^{a}	58.6^{a}	0.05	*
39-44	59.7^{abc}	60.3^{a}	59.7^{ab}	58.7^{c}	59.0^{bc}	59.1^{bc}	59.4^{abc}	59.4^{abc}	59.3^{abc}	59.1^{bc}	0.11	*
27-44	58.3^{a}	58.2^{a}	57.8^{abc}	57.1^{c}	57.4^{bc}	57.8^{ab}	57.9^{ab}	57.9^{ab}	57.9^{ab}	57.9^{ab}	0.08	*

a,b,c :means in the same row bearing different superscripts are significantly different ($p \leq 0.05$).

* Bee bread (B) , bee pollen (P) and combination(BP)

Table (5): Effect of adding of bee bread, bee pollen and their mixtures on egg production and egg mass of Hy-line laying hens

Tre. / Age	C	CP	B 0.1	B 0.15	B 0.2	P 0.1	P 0.15	P 0.2	BP 0.05+0.05	BP 0.1 + 0.1	±SE	Sig.
Egg mass Kg /hen/period												
27-30	1.53	1.54	1.54	1.52	1.48	1.51	1.50	1.52	1.51	1.52	8.88	NS
31-34	1.58^{ab}	1.57^{abc}	1.57^{abc}	1.49^{c}	1.55^{bc}	1.58^{ab}	1.61^{ab}	1.64^{a}	1.66^{ab}	1.55^{bc}	9.52	*
35-38	1.57cd	1.60bcd	1.54bcd	1.56abc	1.52cd	1.5d	1.95ab	1.61a	1.57abc	1.55^{abcd}	0.05	*
39-44	1.66^{b}	1.75^{a}	1.76^{a}	1.74^{a}	1.80^{a}	1.66^{b}	1.78^{a}	1.78^{a}	1.77^{a}	1.72^{ab}	1.03	*
27-44	6.28^{b}	6.40^{ab}	6.41^{ab}	6.31^{ab}	6.35^{ab}	6.25^{b}	6.48^{ab}	6.56^{a}	6.45^{ab}	6.34^{ab}	26.3	*
Feed intake /hen/day												
27-44	100	100	10	100	100	100	100	100	100	100	--	NS
Feed conversion ratio												
27-30	2.10	2.09	2.09	2.11	2.16	2.12	2.14	2.11	2.13	2.12	0.09	NS
31-34	1.96^{bc}	1.98^{abc}	1.98^{abc}	2.08^{a}	2.00^{abc}	1.96^{bc}	1.93^{bc}	1.89^{c}	1.94^{bc}	2.01^{ab}	0.05	0.05
35-38	2.04^{ab}	2.01^{abc}	2.01^{abc}	1.99^{bcd}	2.04^{ab}	2.07^{a}	1.95^{cd}	1.93^{d}	1.98^{bcd}	2.00^{abcd}	0.01	0.05
39-44	2.05^{a}	1.95^{b}	1.93^{b}	1.96^{a}	1.89^{b}	2.05^{a}	1.91^{b}	1.91^{b}	1.92^{b}	1.98^{ab}	0.08	0.05
27-44	2.4^{ab}	2.01^{ab}	2.00^{ab}	2.03^{ab}	2.02^{ab}	2.05^{a}	1.98^{ab}	1.96^{b}	1.99^{ab}	2.03^{ab}	0.06	0.05

a,b,c :means in the same row bearing different superscripts are significantly different ($p \leq 0.05$) NS= Non significant

Table (6): Effect of adding of bee bread, bee pollen and their mixtures on the chemical composition of fresh and stored egg yolks for 21 days at 5°c of Hy-line laying hens at 39 weeks of age

Tr.	Fresh egg yolks			Stored egg yolks		
	Triglyceride (mg/dl)	T. cholesterol (mg/dl)	T. Antioxidant (mM/L)	Triglyceride (mg/dl)	T. cholesterol (mg/dl)	T. Antioxidant (mM/L)
Bee bread(B) , bee pollen(P) and combination(BP) g/kg						
C	212.97^{abc}	77.56^{a}	0.62^{b}	125.94^{ab}	63.79^{ab}	0.82
PC	168.26^{cde}	65.16^{abc}	0.72^{ab}	142.70^{ab}	18.02^{b}	0.90
$B_{0.1}$	256.57^{a}	57.85^{bc}	0.52^{b}	99.25^{b}	92.41^{a}	0.74
$B_{0.15}$	229.32^{ab}	58.24^{bc}	0.75^{ab}	185.57^{a}	35.32^{b}	0.73
$B_{0.2}$	151.34^{de}	74.19^{ab}	0.71^{ab}	205.93^{a}	45.99^{ab}	0.82
$P_{0.1}$	206.05^{abcd}	73.44^{ab}	0.65^{ab}	126.68^{ab}	50.89^{ab}	0.65
$P_{0.15}$	155.92^{de}	71.74^{abc}	0.63^{ab}	188.58^{a}	37.99^{b}	0.70
$P_{0.2}$	181.19^{bcde}	53.68^{c}	0.61^{b}	185.99^{a}	24.72^{b}	0.72
$BP_{0.05+0.05}$	133.50^{e}	61.07^{abc}	0.69^{ab}	99.18^{b}	26.31^{b}	0.79
$BP_{0.1+0.1}$	199.12^{bcd}	56.09^{bc}	0.85^{a}	172.59^{ab}	28.33^{b}	0.82
±SE	7.13	2.06	0.02	9.18	5.32	0.03
Sig.	0.05	0.05	0.05	0.05	0.05	NS

a,b,c :means in the same row bearing different superscripts are significantly different ($p \leq 0.05$).

Table (7): Effect of adding of bee bread, bee pollen and their mixtures on digestibility coefficient of nutrients of Hy-line laying hens at 44 weeks of age

Traits	C	PC	B 0.1	B 0.15	B 0.2	P 0.1	P 0.15	P 0.2	BP 0.05+0.05	BP 0.1 + 0.1	±SE	Sig.
Digestibility coefficient %												
DM	75.92	75.65	75.91	75.95	75.96	75.97c	75.97	75.76	75.65	75.76	0.13	NS
CP	88.06	88.51	88.32	87.49	88.21	87.41	88.25	86.99	87.09	87.05	0.18	NS
EE	53.33^{ab}	51.26^{b}	56.24^{ab}	58.09^{a}	52.68^{ab}	50.76^{b}	57.90^{a}	55.88^{ab}	49.92^{b}	53.68^{ab}	0.72	*
CF	17.37^{bc}	20.82^{abc}	17.62^{bc}	17.12^{bc}	20.23^{abc}	25.51^{a}	21.05^{abc}	23.40^{ab}	23.16^{abc}	24.03^{a}	0.71	*
OM	80.86^{bc}	79.71^{d}	81.11^{abc}	81.84^{ab}	80.64^{cd}	81.60^{abc}	80.80^{bc}	81.13^{abc}	82.01^{a}	81.68^{abc}	0.15	*
NFE	84.90^{ab}	84.34^{ab}	84.13^{ab}	84.92^{ab}	84.15^{ab}	85.61^{a}	83.47^{b}	83.97^{ab}	85.10^{ab}	85.36^{ab}	0.2	*
TDN%	77.65^{abc}	76.51^{c}	78.33^{ab}	77.91^{abc}	77.92^{abc}	76.59^{c}	78.43^{ab}	77.08^{ab}	77.57^{bc}	78.88^{a}	0.19	*

a,b,c :means in the same row bearing different superscripts are significantly different ($p \leq 0.05$). NS= Non significant

Table (8): Effect of adding bee bread, bee pollen and mixtures of them hematology traits of Hy- line laying hens

Tre.	Serum hematology traits						
	RBC*10^6	**WBC*10^3**	**^{1}H%**	**^{2}L %**	**H/L**	**^{3}M%**	**^{4}E**
C	5.83^{ab}	13.33^{d}	14.0^{abc}	80.67^{abc}	0.17^{ab}	4.00^{b}	1.33^{ab}
PC	4.67^{b}	14.67^{cd}	16.67^{abc}	76.33^{abc}	0.22^{ab}	7.00^{ab}	0.0^{b}
$B_{0.1}$	5.15^{ab}	16.0^{bcd}	19.0^{a}	71.5^{c}	0.27^{a}	7.00^{ab}	2.5^{a}
$B_{0.15}$	5.70^{ab}	13.33^{d}	17.33^{abc}	74.33^{bc}	0.25^{ab}	7.33^{ab}	1.00^{ab}
$B_{0.2}$	5.4^{ab}	16.00a^{bcd}	18.00^{ab}	75.00^{abc}	0.24^{ab}	6.67^{ab}	0.33^{b}
$P_{0.1}$	6.1^{ab}	16.67^{abcd}	14.67^{abc}	77.0^{abc}	0.19^{ab}	8.00^{a}	0.33^{b}
$P_{0.15}$	6.4^{ab}	20.67^{a}	14.00^{abc}	77.67^{abc}	0.18^{ab}	8.33^{a}	0.0^{b}
$P_{0.2}$	6.5^{a}	19.33^{ab}	8.67c	85.33^{a}	0.11^{b}	6.00^{ab}	0.0^{b}
$BP_{0.05+0.05}$	6.2^{ab}	15.33^{bcd}	9.33bc	84.33^{ab}	0.11^{b}	4.67^{ab}	1.67^{ab}
$BP_{0.1+0.1}$	6.6^{a}	18.00^{abc}	9.5^{bc}	81.25^{abc}	0.12^{b}	8.25^{a}	1.0^{ab}
±SE	0.18	0.55	0.96	1.11	0.02	0.40	0.20
Sig.	0.05	0.05	0.05	0.05	0.05	0.05	0.05

a,b,c :means in the same row bearing different superscripts are significantly different ($p \leq 0.05$).

Table (9): Effect of adding bee bread, bee pollen and their mixtures on serum biochemical traits of Hy- line laying hens

Tr.	Serum biochemical traits %					
	T. protein (g/dl)	**Albumen(g/dl)**	**Globulin**	**AST (U/ml)**	**ALT (U/ml)**	**T. antioxidant (mM/L)**
C	7.37^{abc}	2.17^{ab}	5.20^{abc}	139.8^{ab}	17.6^{d}	1.31^{bc}
PC	7.71^{ab}	2.28^{ab}	5.43^{ab}	118.6^{ab}	41.6^{a}	1.32^{bc}
$B_{0.1}$	8.46^{ab}	2.00^{b}	6.46^{ab}	106.4^{b}	19.6^{bcd}	1.31^{bc}
$B_{0.15}$	8.19^{ab}	2.52^{a}	5.67^{ab}	108.8^{b}	26.8^{bcd}	1.74^{ab}
$B_{0.2}$	9.20^{a}	2.22^{ab}	6.98^{a}	143.8^{ab}	30.8^{b}	1.81^{a}
$P_{0.1}$	8.40^{ab}	2.21^{ab}	6.19^{ab}	138.4^{ab}	26.4^{bcd}	1.09^{c}
$P_{0.15}$	7.70^{ab}	2.25^{ab}	5.45^{ab}	121.8^{ab}	29.4^{bc}	1.83^{a}
$P_{0.2}$	6.40^{bc}	2.10^{ab}	4.29^{bc}	137.0^{ab}	19.0^{cd}	1.80^{a}
$BP_{0.05+0.05}$	5.45^{c}	2.20^{ab}	3.26^{c}	161.0^{a}	25.2^{bcd}	1.93^{a}
$BP_{0.1+0.1}$	7.58^{abc}	2.33^{ab}	5.25^{abc}	136.8^{ab}	26.4^{bcd}	1.52^{abc}
±SE	1.74	0.29	0.24	4.41	1.38	0.06
Sig.	0.05	0.05	0.05	0.05	0.05	0.05

a,b,c :means in the same row bearing different superscripts are significantly different ($p \leq 0.05$).

Table (10): The effect of feeding different levels of dietary crude fiber during laying period on economic efficiency of egg production from 27-44 wks of age

Items / Tre.		Total feed consumed/ hen (kg)TFC[1]	Feed layer cost/ kg (LE)[2]	Total feed consumed cost/ hen (LE)	Egg number/ hen	Price of one egg (LE)	Total return (LE)	Net return (LE)	EEF (%)[3]
Dietary treatments	C	12.8	3.19	40.83	107.8	0.65	70.07	29.24	71.61
	CP	12.8	3.23	41.34	109.8	0.65	71.37	30.04	72.67
	BB_1	12.8	3.22	41.22	110.9	0.65	72.09	30.85	74.84
	$BB_{1.5}$	12.8	3.24	41.47	110.5	0.65	71.83	29.85	71.98
	BB_2	12.8	3.25	41.57	110.6	0.65	71.89	30.32	72.94
	PP_1	12.8	3.27	41.86	108.1	0.65	70.27	28.41	67.86
	$PP_{1.5}$	12.8	3.31	42.37	111.8	0.65	72.67	30.30	71.51
	PP_2	12.8	3.35	42.88	113.0	0.65	73.45	30.57	71.29
	$BP_{0.5+0.05}$	12.8	3.25	41.55	111.4	0.65	72.41	30.86	74.27
	BP_{1+1}	12.8	3.30	42.24	109.5	0.65	71.18	28.94	68.50
Pooled SEM									1.15
Sig.									NS

[1] TFC= (100g/day/hen * 128 days (from 18/8 /2015 to 23/12/ 2015)/1000

[2] LE= Egyptian pound according to price at the experimental time.

[3] EEF (%) = economic efficiency (%) = (Net return LE /Total feed cost LE) × 100.

[4]Relative EE= Assuming EEF of the control equals 100%

NS=Non significant

REFERENCES

Abd El-Baky, H.H.; F.K. El Baz and G.S.E. Baroty (2008). Evaluation of marine alga ulva lactucal. as a source of natural preservative ingredient. Am.-Eurasian J. Agric. Environ. Sci. 3:434-444.

Ali, M.N.; M.S. Hassan and F.A. Abd El-Ghany (2007). Effect of Strain, Type of Natural Antioxidant and Sulphate Ion on Productive, Physiological and Hatching Performance of Native Laying Hens. International Journal of Poultry Science 6 (8): 539-554.

Aliyzicioglu, Y.; O. Deger; E. Ovali; Y.Barlak,; I. Hosver; Y.Tekelloglu and S.C. Karaman (2005). Effect of Turkish pollen and propolis extracts on respiratory burst for K-562 cell lines. In International Immunopharmacology, vol. 5, 2005, no. 11, p. 1652-1657.

Almas, K.; A.Mahmoud and A.A.Dahlan (2001). comparative study of propolis and saline application on human dentin: a SEM study. Indian Journal Dentist Resourch, New Delhi, v. 12, p. 21-70.

Almeida-Muradian L., Pamplona L., Coimbra S., Barth O., (2005). Chemical composition and botanical evaluation of dried bee pollen pellets, J. Food Compos. Anal. 18, 105–111.

Association of Official Analytical Chemists (AOAC). (1998). Official methods of analysis. 15th Ed. Published by the AOAC., Washington, D.C., USA.

Association of Official Analytical Chemists (AOAC). 2004. Official methods of analysis. 16th ed., Association of Official Analytical Chemists, Washington, DC, USA.

Attia, Y.A.; A.M. Al-Hanoun and F. Bovera 2011b. 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.Phys.Anim.Nutr.95,17–26.

Attia, Y.A., A.M. Al-Hanoun; A.E. Tag El-Din; F.Bovera and E. Shewika 2011. Effect of bee pollen levels on productive, reproductive and blood traits of NZW rabbits.J.Anim.Phys.Anim.Nutr.95,294–303.

Awad,A.L.; M.M. Beshara; A.F. Ibrahim and H. N. Fahim 2013. Effect of using bee bread as a natural supplement on productive and physiological performance of local Sinai hens. Egypt. Poult. Sci. Vol (33) (IV): 889 – 913

Bogdanov, S. 2004. Quality and standards of pollen and beeswax. Apiacta, [S.l.], v. 38, p. 334-341, 2004.

Botsoglou, N.A., P. Florou-paneri, I. Nikolakakis, I. Giannenas, V. Dotas, E.N. Botsoglou and S. Aggelopoulos 2005. Effect of dietary saffron (*Crocus sativus* L. on the oxidative stability of egg yolk. Br. Poult. Sci., 46: 701-707.

Caldwell, C. R. 2003. Alkylperoxyl radical scavenging activity of red leaf lettuce (*Lactuca sativa L.*) phenolics. Journal Agricultural Food Chemistry, Madison, v. 51, n. 16, p. 4589-4595.

Carpes, S.T; G.B. Mourao and M.L. Msson 2009. Chemical composition and free radical scavenging activity of *Apis mellifera* bee pollen from Southern Brazil. Braz. J. Food Technol., v. 12, n. 3, p. 220-229.

Cook, F. W. 1959. Staining fixed preparations of chickens blood cells with combination, May- Green Wald- wrght. Phoxine B stain. Avian Dis. **3**: 272-290.

Damintoti K.; H.Mamoudou; J.Simpore and A.Traore 2005. Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso, African J. Biotechnol. 4, 823–828.

Dorman, H. J. D.; M.Kosar; K. Kahlos; Y.Holm and R. Hilyunen2003. Antioxidant properties and composition of aqueous extracts from Mentha species, Hybrids, Varieties, and Cultivars. Journal Agricultural Food Chemistry, Easton, v. 51, n. 16, p. 4563-4569.

Duncan, D.B. 1955. Multiple ranges and multiple f-test, Biometries 11: 1-42.

Folch, J., Lees, M., Stanley, G.H. Sloane 1957. A simple method for the isolation and purification of the total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Galal, A., A.M. Abd El-Motaal; A.M.H. Ahmed and T.G. Zaki 2008. Productive performance and immune response of laying hens as affected by dietary propolis supplementation.Int.J.Poult.Sci.7,272–278.

Garci, M.; C. Perez-Arquillue; T.Juan; M.I. Juan and A. Herrera 2001. Note: pollen analysis and antibacterial activity of Spanish honeys. Food Science TechnologyInternational, Cambridge, v. 7, n. 2, p. 155-158.

Gebara, E. C. E.; L.A. Lima and M.P.A. Mayer 2002. Propolis antimicrobial activity against periodontopathic bacteria. Brazilian Journal Microbiology, São Paulo, v. 33, n. 4, p. 365-369.

Halliwell, B. 1996b. Vitamin C:L antioxidant or pro-oxidant in vivo? Free Radical

Research 25: 439-454.

Ivanova, D.; Gerova D., Chervenkov, T. and Yankova, T. 2005. Polyphenols and antioxidant capacity of Bulgarian medicinal plants, Journal of Ethnopharmacology 69: 145-150.

Jakobsen, P.E.; S.G. Kirston and S.H. Nilson 1960. Digestibility trials with poultry. 322 Bereting fraforsgs

Laboratoriet udgivet of tants. Husdyrbugsud Valy - Kaben Haven

Joseph, J.A; B. Shukitt-Hale; N.A. Denisova; D.Bielinski; A. Martin; J.J. McEwen and P.C. Bickford 1999. Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberries, spinach, or strawberry dietary supplementation. J. Neurosci. 19, 8114-8121.

Kang,Y. H.; K.K.Kyoung ; W.K. Tae ; S.Y.Chun and C. Myeon (2015). Evaluation of the Anti-obesity Activity of Platycodon grandiflorum Root and Curcuma longa Root Fermented with Aspergillus oryzae. KOREAN J. FOOD SCI. TECHNOL. Vol. 47, No. 1, pp. 111~118.

Koracevic, D.; G. Koracevic; et al., (2001). Cli. Pathol. 54, 356-361.

Krauss, R.; R. Deckelbaum ; V.Ernst; E. Fisher; B. Howard; R. Knopp; T. Kotchen; A. Lichtenstein; H. McGill; T. Pearson; T. Prewitt; N. Stone; H.L. Van and R. Weinberg (1996). Dietary guidelines for healthy American adults: a statement for health professionals from the nutrition committee American Heart Association. Circulation 94: 1795-1800.

Krell R. 1996. Value-added products from beekeepings, FAO Agric. Serv. 124, 87–113.

Leja, M.; A. Mareczek; G. Wyzgolik; J. Klepacz and K. Czekońska 2007. Antioxidative properties of bee pollen in selected plant species.Food Chem. 100,237–240.

Lichtenstein, A.H; L.J. Appel; M. Brands; M. Carnethon; S. Daniels; H.A. Franch; B. Franklin; P. Kris-Etherton; W.S. Harris; B. Howard; N. Karanja; M. Lefevre; L. Rudel; F. Sacks; H.L. Van; M. Winston and J. Wylie-Rosett 2006. Diet and Lifestyle Recommendations Revision 2006. Circulation 114(1): 82-96.

Lobo, V.; A. Patil, ; A. Phatak and N.Chandra 2010. Free radicals, antioxidants and foods: impact on human health. Pharmacol. Rev. 4:118-126.

Markham, K.E.; K.A.Mitchel; A.L.Wilkins; J.A. Daldy; Y. Lu (1996). HPLC and GC–MS identification of the major organic constituents in New Zealand propolis. Phytochemistry42, 205–211.

Miura, Y. T.; L. Chiba; L.Tomita; H. Koizumi; S.Miura; K.Umegaki; Y.Hara; M.Ikeda; and T. Tomita 2001. Tea catechins prevent the development of atherosclerosis in apoprotein E-deficient mice. J. Nutr. 131:27-32.

Nagaia,T; T. Nagashimaa; N. Suzukib, and R. Inoue 2005. Antioxidant Activity and Angiotensin I-Converting Enzyme Inhibition by Enzymatic Hydrolysates from Bee Bread. Z. Naturforsch. 60c, 133-138.

Natt, M. P.; and C.A. Hrrick 1952. A new blood diluent for counting the erythrocytes and leukocytes for the chickens. Poultry Science **31**: 1492-1496.

Nijveldt R.; E. Nood; D. Hoorn; P. Boelens; K. Norren and P. Leeuwen 2001. Flavonoids: A review of probable mechanisms of action and potential applications, Am. J. Clin. Nutr. 74, 418–425.

Panda, A.K.; M.R. Reddy; S.V. Ramarao and N.K. Praharaj 2003. Production

performance, serum/yolk cholesterol and immune competence of White Leghorn layers as influenced by dietary supplementati on with probiotic. Tropical Animal Health and Production 35: 85-94.

Pelletier, X; P. Thouvenot; S. Belbraouet; J. Chayvialle; B. Hanesse; D. Mayeux and G. Debry 1996. Effect of egg consumption in

healthy volunteers: influence of yolk, white or whole-egg on gastric emptying and on glycemic and hormonal responses. Ann Nutr Metab 40: 109-115.

Peric, L; D. Žikic and M. Lukic 2009. Application of alternative growth promoters in broiler production. In Biotechnology in Animal Husbandry. vol. 25, 2009, p. 387-397.

Popiela-Pleban , E. R.; Z. Dobrzanski; K. Pogoda-Sewerniak; S. Opalinski and M. Korczynski 2012. Effect of propolis and bee pollen supplementation on selected blood parameters of laying hens .In: Book of Abstracts, World's Poult. Sci..(Suppl.1)659.The 24th World Poultry Congress August5–9, Salvador ,BA, Brazil.

Proestos, C.; N. Chorianopoulos; G.J.E. Nichas and M. Komaitis 2005.RP-HPLC analysis of the phenolic compounds of plant extracts: investigation of their antioxidant capacity and antimicrobial activity. JournalAgricultural Food Chemistry, Easton, v. 53, n. 4, p. 1190-1195, 2005.

Quian, W.L; Z. Khan; D.G. Atson and J. Fearnley 2008. Analysis of sugarsin bee pollen and propolis bylig combination with pulsedam pero metric detection and mass spectrometry. J.FoodCompos.Anal.21,78–83.

Radwan, N. L.; R.A. Hassan; E.M. Qota and H.M. Fayek 2008. Effect of Natural Antioxidant on Oxidative Stability of Eggs and Productive and Reproductive Performance of Laying Hens. Int. J. of Poult. Sci. 7 (2): 134-150.

Raederstorf, G.F.; M.F. Schlachter; V. Elste and P. Weber (2003). Effects of green tea supplementation on lipid absorption and plasma lipid levels in rats. J. Nutr. Biochem. 14:326-329.

Richmond, W. 1973. Preparation and properties of a cholesterol oxidase from Nocardia sp. And its application to the enzymatic assay of total cholesterol in serum. Clin. Chem. **19**: 1350-1356.

Sacchetti, G.; S. Maietti; M. Muzzoli; M. Scaglianti; S. Manfredini and M. Radice 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chemistry, 91(4), 621–632.

Šarić A.; T. Balog; S. Sobočanec ; B. Kušić ; V. Šverko; G. Rusak; S.,Likić; D. Bubalo; B. Pinto; D. Reali and T. Marotti 2009. Antioxidant effects of flavonoid from Croatian *Cystus incanus* L. rich bee pollen. Food Chem Toxicol 47, 547-554.

Schnohr, P; O. Thomsen; H.P. Riis; G. Boberg-Ans; H. Lawaetz and T. Weeke 1994. Egg consumption and high-density lipoprotein cholesterol. J Intern Med 235: 249-251.

SPSS. (2008). SPSS User´s Guide Statistics. Ver. 17. Copyright SPSS Inc., USA.

SU, M. and J.L. SILVA 2006 Antioxidant activity, anthocyanins, and phenolics of rabbiteye blueberry (*Vaccinium ashei*) by-products as affected by fermentation. Food Chem., v.97, p.447-451.

Turrens, J.F; J.D. Crapo and B.A. Freeman 1984. Protection against oxygen toxicity by intravenous injection of liposome-entrapped catalase and superoxide dismutase. Journal of Clinical Investigation **73:** 87-95.

Vasudevan, D.M. and S. Sreekumari 2001. Free radicals and antioxidants. In "Textbook of biochemistry for medical students". pp 212- 215. 3rd edition. Jaypee Brothers Medical Publishers(P) Ltd, New Delhi.

Wang, J.; S. Li.; Q. Wang B. Xin and H. wang 2007. Trophic effect of bee pollen on small intestine in broiler chickens. J. Med. Food 10 (2) 276-280.

Youdim, K.A.; B. Shukitt-Hale; S. MacKinnon; W. Kalt and J.A. Joseph,

(2000). Polyphenolics enhance red blood cell resistance to oxidative stress: in vitro and in vivo. Biochim. Biophys. Acta 1523, 117-122.

Young, I.S. and J.V. Woodside 2001. Antioxidants in health and disease. J. Clin. Pathol. 54:176-186.

Yu, L. L.; K. K. Zhou and J. Parry 2005. Antioxidant properties of cold pressed black caraway, carrot, cranberry and hemp seed oils. Food Chem., 91: 723-729.

Xu, X; L. Sun; J. Don and H. Zhang (2009). Breaking the cells of rape bee pollen andconsecutive extraction of functional oil with supercritical carbon oxide. In Innovative Food Science and Emerging Technologies, vol. 10, 2009, p. 42-46.

Zuluaga,C.M.; C. Juan ; M. Serrato and C. Quicazan.2015. Chemical, nutritional and bioactive Ccharacterization of Colombian bee-bread. Chemical Engineering Transactions, 43, 175-180 DOI: 10.3303/CET1543030.

الملخص العربي

تأثير اضافة خبز النحل وحبوب اللقاح كمضادت أكسدة علي الأداء الإنتاجي والخصائص الوظيفية للدجاج البياض سلالة الهاي- لاين

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الهدف من هذا البحث هو دراسة تأثير اضافة خبز النحل وحبوب اللقاح والخليط بينهما علي الأداء الإنتاجي والخصائص الوظيفية لبيض المائدة لدجاجات سلالة الهاي- لين. تم استخدام ما جملتة 1000 دجاجة من سلالة الهاي-لين البياضة علي عمر 27 اسبوع وقد تم وزنها وتوزيعها عشوائيا الي عشرة معاملات تجريبية لكل معاملة 100 دجاجة ولكل معاملة 5 مكررات (20دجاجة / مكرره) وقد اشتمل تصميم التجربة علي عشرة معاملات غذائية وهي كما يلي: عليقة بياض مقارنة– عليقة مقارنة موجبة تحتوي علي 0.015% BHT– وباقي المعاملات التجريبية قسمت الي ثماني مجموعات اضيف اليها مستويات متدرجة من خبز النحل (0.1- 0.15- 0.2%) ومستويات متدرجة من حبوب اللقاح (0.1- 0.15- 0.2%) وخليط من خبز النحل وحبوب اللقاح (0.05+ 0.05% و 0.1+ 0.1%). أوضحت النتائج أن كل العلائق التجريبية حققت عدد من البيض الناتج اعلي من العليقة المقارنة لكن لوحظ تحسن واضح في عدد البيض الناتج من الدجاجات المغذاه علي عليقة مضاف اليها 2جم حبوب لقاح / كجم عليقة ويلها من حيث التحسن في عدد البيض العلائق المضاف البها 1.5 جم حبوب لقاح و0.5جم خبز النحل+0.5جم حبوب لقاح. تحسنت كتلة البيض الناتج معنويا بالتغذية علي العليقة المضاف اليها 2 جم حبوب لقاح / كجم عليقة مقارنة بالعليقة المقارنة. سجلت العلائق المضاف اليها 2جم جبز النحل و1.5 جم حبوب اللقاح و 1جم جبز النحل قيما أقل بدرجة معنوية في الجليسيريدات الثلاثية في صفار البيض الطازج مقارنة بالعليقة المقارنة. فيما يتعلق بمستوي الكولستيرول في صفار البيض المخزن للبيض الناتج من الدجاجات المغذاه علي العلائق المضاف اليها 150مجم BHT و 2حم حبوب اللقاح و1جم خبز النحل+1جم حبوب اللقاح 1.5جم خبز النحل و 1.5 جم حبوب اللقاح و2 جم خبز النحل سجلت قيما تتراوح بين 18.02و 45.99 مجم/ ديسي لتر بينما بلغت تلك القيمة في صفار بيض عليقة المقارنة 63.79 مجم/ ديسي لتر. تحسن معامل هضم المستخلص الإيثيري معنويا نتيجة التغذية علي العليقة المحتوية 1.5 جم خبز النحل و 1.5 جم حبوب لقاح / كجم عليقة مقارنة بالعليقة المقارنة. أظهرت الدجاجات التي تم تغذيتها علي عليقة بها 0.5جم خبز النحل+0.5جم حبوب لقاح /كجم عليقة و 0.2% خبز النحل و 0.15% جم حبوب اللقاح و0.2% حبوب اللقاح قيم لمضادات الأكسدة الكلية في سيرم الدم أعلي معنويا مقارنة بالعليقة المقارنة. وفي الواقع فإن دجاجات الهاي لين التي تم تغذيتها علي 0.2% خبز النحل و0.15% جم خبز النحل و0.1% حبوب اللقاح و0.1% خبز النحل عليقة يمكن أن تستخدم كمصدر لمضادات الأكسدة لدعم صحة الطيور والأداء الإنتاجي وأيضا جودة البيض الداخلية هو احتياج أساسي فيما يخص صحة المستهلك و هو ما يعرف بالمنتجات العضوية.