

## EGG PRODUCTION PERFORMANCE, BLOOD BIOCHEMICAL AND IMMUNOLOGICAL RESPONSE OF LAYING JAPANESE QUAIL FED DIET SUPPLEMENTED WITH PROPOLIS AND BEE POLLEN

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### SUMMARY

**I**ncreasing productivity, improving product quality and insuring animal welfare are general demands to animal breeders. The aim of this study was to evaluate the effect of supplementing the diet with propolis and/or bee pollen on egg productivity and immunological responses of laying Japanese quail. A total of 200 female quail, 35 days old, were randomly allocated to four equal groups. The first group served as a control group and was fed a basal diet (C). The second group was fed the basal diet supplemented with propolis at 1g/kg diet (Pro). The third group was fed the basal diet supplemented with bee pollen at 2g/kg diet (Bp). The fourth group was fed the basal diet supplemented with both propolis (1g/kg diet) and bee pollen (2g/kg diet) (Pro+Bp). Feed intake, feed conversion, egg mass, shell thickness and yolk index were significantly improved due to different dietary supplementation. Blood total protein and albumin increased, whereas cholesterol and H/L ratio significantly decreased due to diet supplementation. A synergistic effect of Pro+Bp was noticed in blood albumin, calcium and H/L ratio. In conclusion propolis and bee pollen can be supplemented to the diet of laying quail to increase egg production, improve egg quality and enhance blood protein, lipid and immunological responses.

**Keywords:** *propolis, bee pollen, quail, production performance, egg quality, blood parameters.*

### INTRODUCTION

The Japanese quail (*Coturnix coturnix japonica*) has relative advantages compared to other poultry species. These advantages have been summarized as: 1) Dual purpose poultry species producing eggs and meat, 2) Low maintenance cost with short generation interval, high egg production and diseases resistance, 3) Japanese quail is the smallest avian species farmed for meat and egg (Vali, 2008 and Mohammed and Ejiofor, 2015). Quail egg yolk has the highest monounsaturated fatty acid (MUFA) reaching 51.6% and 12% polyunsaturated fatty acid (PUFA) compared to other wild birds maintained in captivity (Choi *et al.*, 2001). Al-Obaidi and Al-Shadeedi (2017) found no significant differences between chicken and quail egg in albumin content (11.76 vs. 11.80%) and egg yolk content (17.59 vs. 15.58%), respectively. They also found that quail egg cholesterol and LDL (mg/g) levels were significantly lower than in the chicken egg. These findings imply that the quail egg is relatively healthier than the chicken egg especially for people suffering from cardiac problems. Loetscher *et al.* (2014) reported a significant transfer of antioxidants from the diet supplemented with antioxidants to the hen egg, and they claimed that using natural plant-origin additives with antioxidant properties is suggested to support eggs with relatively high antioxidant properties. Therefore, the high concentrations of MUFA and PUFA in quail egg require powerful antioxidant supplementation to protect these fatty acids from oxidation. Poultry feed must be formulated to provide all the nutrients necessary for growth and health. The naturally available growth factors, probiotics and antimicrobial peptides, ought to be used due to their lower side effects on bird wellbeing and the health status of the consumers (Keerthana and Renuka, 2017). Propolis and bee pollen were suggested as natural growth-

promoter substitutions of antibiotics in poultry diets (Kročko *et al.*, 2012; Babaei *et al.*, 2016; Haščík *et al.*, 2017; Khan, 2017). Propolis is a resin substance collected from plant by honeybees (*Apis mellifera*) and is used in disease resistance inside bee hives (Simone-Finstrom and Spivak, 2010). It has a wide range of biological effects such as immunomodulatory, anti-microbial, anti-viral, anti-fungal, anti-inflammatory and anti-tumor activities (Lotfy, 2006; Viuda-Martos *et al.*, 2008). Bee pollen is another product of honeybees that is collected for the purpose of feeding their larvae in the early stages of development and is considered the main source of proteins, minerals and fats (Campos *et al.*, 2008). On the other hand, bee pollen is composed of different bioactive substances such as essential amino acids, lipids, glucosinolates, phenolic compounds, vitamins and minerals that make it an attractive natural healthy food (Roulston and Cane, 2000; Ares *et al.*, 2018). The antimicrobial, antimutagenic, antioxidant and anti-inflammatory activities of bee pollen are well documented (Pascoal *et al.*, 2014). Broad scopes of positive immunological, histological and productive effects were observed when propolis and/or bee pollen were supplemented to poultry diet. Increasing feed intake, body weight gain and improving feed conversion ratio were common effects of the use of propolis and/or bee pollen supplementation in broiler, quail and rabbit diets (Attia *et al.*, 2011ab; Hosseini *et al.*, 2016; Mehaisen *et al.*, 2017). The immunological status of broiler chicken was enhanced with bee pollen supplementation (Oliveira *et al.*, 2013). Research investigations studied the effects of propolis and bee pollen supplementation on alleviation the negative effect of heat stress oxidative damage in growing and laying quail. Significant effects of propolis and bee pollen supplementation to quail diet on the productive performance, immunological response and blood lipid profile were reported (Zeweil *et al.*, 2016; Mehaisen *et al.*, 2017). The present study aimed to investigate the effects of propolis and bee pollen supplementation to the diet on productive performance, egg quality, blood constituents and immunity parameters of laying Japanese quail reared under thermo-neutral environment and to find out if there is a synergistic effect between the two supplementations on quail productive performance.

## **MATERIALS AND METHODS**

### ***Birds management:***

A total of 200 female Japanese quail, 35 days old, were allocated randomly to four equal groups. Birds were distributed into 20 wired-cages [10 birds per cage, measured 60 × 50 × 50 cm for length, width and height, respectively] in an environmentally-controlled room. The brooding temperature was daily recorded three times at 8 a.m. 3 p.m. and 8 p.m. using digital thermometer and was found to be ranged from 25.4±0.5 to 27.3±0.4°C. The relative humidity was 50 to 60%. Lighting was set to provide birds with 16 h light and 8h dark daily throughout the experiment.

### ***Experimental design:***

The birds were randomly divided into four groups. The first group was fed the basal diet without any supplementation and served as the control (C). The second group was fed the basal diet supplemented with propolis at 1g/kg diet (Pro). The third group was fed the basal diet supplemented with bee pollen at 2g/kg diet (Bp). The fourth group was fed propolis (1g/kg) and bee pollen (2g/kg), (Pro+Bp). Feeding regime was started at 35 days of age and ended at 84 days of age. The first seven days were set as adaptation period and the parameters were taken starting from 42 to 84 days of age

### ***Egg production and egg quality:***

The initial and final body weights (g) were individually recorded at the beginning and at the end of the experiment (42 and 84 d of age). Egg number and egg weight were recorded daily, and egg mass was calculated for each group. The feed intake (g/bird/d) was measured for each treatment group. The feed conversion ratio was calculated for each treatment.

At the end of the experiment, 15 eggs were chosen randomly from each group. Egg weight was measured to the nearest 0.1g. Egg width and egg length (cm) were calibrated using an electronic digital device. Egg shape index was calculated according to Das *et al.* (2010) as: (egg width/egg length) × 100.

Eggshell thickness (mm) was measured once the egg shell was dried at room temperature, recorded as the average of estimates from both ends and the equator of the examined eggs (Das *et al.*, 2010). Eggshell weight (g) was determined after the shell had been dried at room temperature. Egg shell ratio was calculated as: (shell weight/egg weight)  $\times 100$ .

Internal egg quality parameters were also measured. The yolk was gently separated from the albumin and weighed. The albumen mass was calculated by subtracting the shell and yolk weights from the total egg weight. The yolk and albumen dimensions (mm) were estimated according to Reddy *et al.* (1979). Yolk index was calculated as: [yolk height (cm)/yolk diameter (cm)]  $\times 100$ . Yolk ratio was calculated as: (yolk weight (g)/egg weight (g)  $\times 100$ ). Albumen ratio was computed as: [albumen weight (g)/egg weight (g)]  $\times 100$  (Romanoff and Romanoff, 1949). The Haugh units were determined using the following formula:  $\log [\text{albumen height (mm)} + 7.57 - 1.7 \times (\text{egg weight (g)})^{0.37}] \times 100$ , (Haugh, 1937).

The birds were fed a basal diet (Table 1) according to NRC (1994) guideline. The diet and fresh water were provided *ad libitum*.

**Table (1): Basal diet composition and calculated analysis.**

Ingredient	%
Yellow corn	64.50
Soybean meal, 44%	20.50
Protein concentrate, 52%	10.00
Di-calcium phosphate	2.31
Limestone	0.96
Lysine	0.08
DL-methionine	0.09
Premix	1.06
Vitamin and trace mineral	0.40
Coccidostate	0.10
<i>Calculated analysis</i>	
ME (kcal/kg)	2853
Crude protein, %	20.00
Calcium, %	2.33
Available phosphorus, %	0.66
Lysine, %	1.04
Methionine, %	0.52

***Blood sampling and analysis:***

Twelve blood samples from each experimental group were collected biweekly in two heparinized tubes. One tube was centrifuged at  $2000 \times g$  for 10 min at  $4^\circ\text{C}$ , and plasma was separated and stored at  $-20^\circ\text{C}$  until further analysis. The plasma total protein, albumin, cholesterol, triglycerides, calcium, phosphorus, AST and ALT were measured in plasma using kits (Salucea, Haansberg, Netherlands).

The other heparinized tube was assigned to measure the total white blood cells (TWBCs) and the heterophil/lymphocyte (H/L) ratio. The TWBCs were manually determined by mixing  $490\mu\text{l}$  of brilliant cresyl blue dye with  $10\mu\text{l}$  of the whole blood sample, and then the total leukocytes were counted under a microscope at a magnification of  $200\times$  using a hemocytometer slide (Gehad *et al.*, 2008). The H/L ratio was determined using Hema-3 stain (cat# 22-122911, Fisher scientific, USA), according to Zhang *et al.*, (2009).

**Statistical analysis:**

Statistical analysis was performed using the general linear model (GLM) procedure of SAS (2006) considering the treatment effect. Differences among means were obtained using Duncan's new multiple range test at  $P < 0.05$  (Duncan, 1955).

**RESULTS AND DISCUSSION****Production performance:**

The production performance parameters are presented in Table (2). The mean initial and final body weight were  $252 \pm 1$  and  $342 \pm 1$  g, respectively, and the differences between experimental groups were insignificant. Feed intake was significantly increased ( $P < 0.0001$ ) in all the supplemented groups by about 11% compared to the control group. Feed conversion was improved by about 13% when diet was supplemented with Pro, Bp or both Pro+Bp. These results can be explained by the reported effects of propolis and bee pollen on increasing the crypts depth in quail ileum (Mehaisen *et al.*, 2017) and in broiler jejunum (Hosseini *et al.*, 2016). Moreover, Wang *et al.* (2007) reported that when bee pollen was supplemented to the diet of broiler chickens, a trophic effect was shown by increasing thickness and length of the small intestine villi. These findings have a direct effect of propolis and bee pollen on increasing nutrients absorption and consequently improving feed conversion ratio. Another explanation was suggested by Kročko *et al.* (2012) that propolis and bee pollen supplementations modulated the bacterial colonization pattern in favor to beneficial microorganisms in broiler chickens.

**Table (2): Performance and egg production parameters of Japanese quail supplemented with propolis (Pro), bee pollen (Bp) or both (Pro+Bp).**

Item	C	Pro	Bp	Pro+Bp	P-value
Initial body weight, g	249 $\pm$ 2	252 $\pm$ 3	251 $\pm$ 2	255 $\pm$ 2	0.354
Final body weight, g	340 $\pm$ 2	343 $\pm$ 2	340 $\pm$ 2	345 $\pm$ 2	0.277
Feed intake, g/bird/d	21.27 $\pm$ 0.12 <sup>b</sup>	23.68 $\pm$ 0.02 <sup>a</sup>	23.73 $\pm$ 0.09 <sup>a</sup>	23.48 $\pm$ 0.11 <sup>a</sup>	0.0001
Feed conversion	3.45 $\pm$ 0.41 <sup>a</sup>	2.97 $\pm$ 0.28 <sup>b</sup>	3.06 $\pm$ 0.28 <sup>b</sup>	2.94 $\pm$ 0.28 <sup>b</sup>	0.006
Egg number, bird/d	0.52 $\pm$ 0.06 <sup>b</sup>	0.64 $\pm$ 0.06 <sup>a</sup>	0.63 $\pm$ 0.05 <sup>ab</sup>	0.64 $\pm$ 0.06 <sup>a</sup>	0.0001
Egg weight, g	12.16 $\pm$ 0.25 <sup>b</sup>	12.59 $\pm$ 0.12 <sup>a</sup>	12.44 $\pm$ 0.20 <sup>ab</sup>	12.59 $\pm$ 0.12 <sup>a</sup>	0.027
Egg mass, d	6.36 $\pm$ 0.80 <sup>c</sup>	8.13 $\pm$ 0.79 <sup>a</sup>	7.89 $\pm$ 0.70 <sup>b</sup>	8.16 $\pm$ 0.81 <sup>a</sup>	0.0001

Means within the same row with different superscripts differ significantly ( $P < 0.05$ ).

On the other hand, egg production (number of eggs per day) was significantly increased with propolis supplementation, with or without bee pollen, compared to the control group. Also, egg weight showed a significant increase in the Pro and Pro+Bp groups and a tendency of increasing egg weight in the Bp group. These results are closely related to the high feed intake and improved feed conversion that were observed in each of the supplemented groups.

**Egg quality**

The internal and external egg quality parameters are presented in Table (3). The supplementation of diet with Pro, Bp and Pro+Bp resulted in significant increases in shell thickness and yolk index compared to the control. The significant increases in shell weight and shell percentage were prominent in the Pro and Pro+Bp groups. The egg quality parameters are all in agreement with the result of El-Tarabany (2016) in Japanese quail. It can be implied that the observed differences in yolk parameter due to treatment can be partially attributed to the increasing level of sexual hormones, estrogen and progesterone as reported by Attia *et al.*

(2011a) in rabbits fed bee pollen in diet. Estrogen increases the production of yolk precursors in the liver and results in changes in the calcium bone mobilization facilitating eggshell production.

**Table (3): Egg quality parameters of Japanese quail supplemented with propolis (Pro), bee pollen (Bp) or both (Pro+Bp).**

Egg Quality Parameters	C	Pro	Bp	Pro+Bp	P-value
Egg weight, g	12.87±0.22	13.27±0.37	13.00±0.34	13.27±0.23	0.714
Shape index %	78.99±0.87	79.62±1.17	79.44±0.63	79.80±0.80	0.928
Shell weight, g	1.07±0.02 <sup>b</sup>	1.18±0.03 <sup>a</sup>	1.13±0.04 <sup>ab</sup>	1.19±0.02 <sup>a</sup>	0.007
Shell %	8.04±0.19 <sup>b</sup>	8.97±0.29 <sup>a</sup>	8.83±0.40 <sup>ab</sup>	8.99±0.23 <sup>a</sup>	0.043
Shell thickness, mm	0.27±0.004 <sup>b</sup>	0.29±0.004 <sup>a</sup>	0.30±0.005 <sup>a</sup>	0.30±0.004 <sup>a</sup>	0.0001
Yolk weight, g	4.46±0.16	4.67±0.16	4.55±0.14	4.67±0.13	0.720
Yolk %	34.65±1.03	35.44±1.37	35.30±1.31	35.29±1.03	0.967
Yolk index, %	38.83±0.10 <sup>b</sup>	42.27±1.07 <sup>a</sup>	41.95±0.70 <sup>a</sup>	42.29±0.51 <sup>a</sup>	0.013
Albumen weight	7.35±0.18	7.42±0.38	7.31±0.36	7.41±0.23	0.992
Albumen, %	57.04±0.10	55.61±1.46	55.86±1.51	55.73±1.15	0.855
Haugh Unit	85.57±0.95	86.28±0.66	85.35±1.39	86.44±0.77	0.829

*Means within the same row with different superscripts differ significantly (P<0.05).*

#### **Blood biochemistry and immune parameters**

Plasma biochemical and immunological parameters of mature female quail supplemented with Pro and/or Bp are presented in Table (4). Dietary supplementation significantly increased plasma total protein, albumin and globulin level (P<0.05). A synergistic effect was noticed in plasma albumin level when comparing Pro+Bp group with the other supplemented and non-supplemented control groups.

Plasma calcium was increased in the Pro+Bp group compared to the control group (P<0.05). The tendency of increasing plasma calcium level in the Pro and Bp groups and the significant increase of plasma calcium in Pro+Bp group may indicate the improvement of calcium metabolism due to the Pro and Bp supplementations, and in turn, significant high shell weight, thickness and percentage were observed (Table 3). The mineral elements of bee pollen showed a predominance of K, P, Ca, and Mg (Bonvehí and Jordà, 1997). Significant reduction in AST activity was observed in supplemented groups compared to the control group indicating positive effect on liver function and activity.

**Table (4): Plasma biochemistry and immunological parameters of Japanese quail supplemented with propolis (Pro), bee pollen (Bp) or both (Pro+Bp).**

Blood parameter	Control	Pro	Bp	Pro+Bp	P-value
Total protein, g/dl	4.37±0.30 <sup>b</sup>	5.87±0.16 <sup>a</sup>	5.80±0.26 <sup>a</sup>	6.12±0.16 <sup>a</sup>	0.0001
Albumin g/dl	2.22±0.10 <sup>c</sup>	2.78±0.10 <sup>b</sup>	2.64±0.15 <sup>b</sup>	3.12±0.09 <sup>a</sup>	0.0001
Globulin, g/dl*	2.15±0.30 <sup>b</sup>	3.08±0.16 <sup>a</sup>	3.16±0.34 <sup>a</sup>	3.00±0.20 <sup>a</sup>	0.030
Calcium, mg/dl	8.75±0.41 <sup>b</sup>	10.11±0.54 <sup>ab</sup>	9.97±0.50 <sup>ab</sup>	10.57±0.61 <sup>a</sup>	0.0475
Phosphorus, mg/dl	6.41±0.27	6.77±0.36	6.60±0.28	6.84±0.28	0.746
AST, U/l	24.17±1.95 <sup>a</sup>	17.94±2.10 <sup>b</sup>	17.24±1.62 <sup>b</sup>	16.32±1.84 <sup>b</sup>	0.050
ALT, U/l	12.14±1.08	12.02±1.30	12.17±1.19	11.99±1.01	0.999
Cholesterol, mg/dl	202±8.56 <sup>a</sup>	170±5.91 <sup>b</sup>	170±4.37 <sup>b</sup>	160±6.01 <sup>b</sup>	0.0002
Triglycerides, mg/dl	218±8.91 <sup>a</sup>	186±10.30 <sup>b</sup>	192±9.01 <sup>ab</sup>	183±10.87 <sup>b</sup>	0.045
TWBCs, ×10 <sup>3</sup> /mm <sup>3</sup>	109±5.49	113±4.77	107±4.60	114±6.51	0.798
H/L ratio	0.46±0.01 <sup>a</sup>	0.32±0.01 <sup>bc</sup>	0.34±0.02 <sup>b</sup>	0.30±0.01 <sup>c</sup>	0.0001

*Means within the same row with different superscripts differ significantly (P<0.05).*

\*: globulin was calculated by subtracting plasma albumin from total protein.

Cholesterol level was significantly decreased due to Pro and Bp supplementation. The combination of Pro and Bp supplementation tended to show a further reduction in cholesterol level compared to each supplementation alone (160 vs. 170 mg/dl, respectively). Triglycerides levels were also significantly decreased in the Pro and Pro+Bp groups. Similar significant reduction in serum cholesterol and triglycerides in broiler chickens fed propolis and/or bee pollen continuously or intermittently were obtained by Attia *et al.* (2014). Also, propolis supplementation to broiler chickens reduced cholesterol and triglycerides when fed at 0.8 g/kg diet (Zafarnejad *et al.*, 2017). The reduction in serum cholesterol and triglycerides were also reported by Farag and El-Rayes (2016) in broilers chicks fed bee pollen, demonstrating negatively related dose depended pattern. Therefore, the significance of reduction in triglycerides in Bp group can be attributed to the dose given. The observed modulation in plasma lipid profile might be related to the high level of PUFA found in both bee pollen and propolis (Shinohara *et al.*, 2002; Xu *et al.*, 2009; Rebiai *et al.*, 2017). Moreover, Tatlı Seven *et al.* (2016) reported a significant increase in tissue PUFA of quail fed bee pollen at 1g/kg diet.

The total white blood cells (TWBCs) did not differ due to diet supplementation. Nevertheless, blood heterophil: lymphocyte ratio (H/L) is an index of oxidative stress. Quail groups supplemented with Pro and Bp had lower H/L ratio by 30 and 26%, respectively, compared to the control group. The Pro+Bp supplementation showed further reduction of H/L ratio reached to 35%. A consistent result on H/L ratio reduction was reported by Hosseini *et al.* (2016), when propolis and bee pollen were supplemented to heat stressed broiler chickens. Moreover, Abass *et al.* (2017) reported a significant reduction in H/L ratio in turkey poults subjected to oxidative stress induced by paraquat injection and feed propolis compared to un-supplemented stressed birds. Although, no significant difference was found when propolis supplemented to unstressed bird compared to the control. This conflicted result could be contributed to the difference in bird species and the production type.

## CONCLUSION

The present study revealed that propolis and bee pollen supplementation, at the level of 1g/kg and 2g/kg diet, respectively; to laying quail diet improved the production performance and ameliorated blood constituent levels. A synergistic effect was observed in plasma albumin, calcium and H/L ratio. Propolis supplementation with or without bee pollen has the most pronounced effect on increasing egg number, egg weight and reducing plasma triglycerides levels. We recommend supplementing the diet of laying quail with propolis and/or bee pollen to improve productivity, enhance egg quality and ameliorate the immune response of laying quail.

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### إنتاج البيض ومكونات الدم والأداء المناعي للسمان الياباني البياض المغذى على عليقة مضاف إليها البروبوليز وحبوب اللقاح

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تعتبر زيادة الإنتاج ورفع جودة المنتج وتحسين حيوية الطيور من المتطلبات العامة للمربين. استهدفت الدراسة تقييم تأثير إضافة صمغ النحل (البروبوليز) وحبوب اللقاح لعلائق السمان البياض على إنتاج البيض ورد الفعل المناعي. تم استخدام عدد 200 من إناث السمان على عمر 35 أسبوع وتم توزيعهم عشوائياً على أربع مجموعات متماثلة. المجموعة الأولى غذيت على العليقة الأساسية بدون أى إضافات (الكنترول)، المجموعة الثانية غذيت على العليقة الأساسية مضاف إليها 1 جم صمغ النحل لكل كجم عليقة (البروبوليز)، المجموعة الثالثة غذيت على العليقة الأساسية مضاف إليها 2 جم حبوب لقاح لكل كجم عليقة (حبوب اللقاح)، والمجموعة الرابعة غذيت على العليقة الأساسية مضاف إليها 1 جم صمغ النحل مع 2 جم حبوب لقاح لكل كجم عليقة (البروبوليز+حبوب اللقاح). عملت جميع الإضافات الغذائية على تحسين كل من كمية الغذاء المأكل وكفاءة تحويل الغذاء وكتلة البيض المنتج وسمك قشرة البيض ودليل الصفار بشكل معنوي. كما ارتفع البروتين الكلي والألبومين في الدم بينما إنخفض كوليستيرول الدم ونسبة الهبتيروفيل إلى الليمفوسيت بشكل معنوي نتيجة للإضافات الغذائية المستخدمة. لوحظ وجود تكامل بين البروبوليز وحبوب اللقاح عند إضافتهم سوياً على كل من البيومين وكالسيوم الدم ونسبة الهبتيروفيل إلى الليمفوسيت. ومن هنا يمكن استنتاج أن إضافة كل من البروبوليز وحبوب اللقاح إلى علائق السمان البياض يعمل على زيادة إنتاج البيض وتحسين خواص البيض المنتج وتحسين بروتين ودهون الدم كما يعمل أيضاً على تحسين رد الفعل المناعي.