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Research Article
Effect of Bee-pollen Supplementation on Performance, Carcass Traits and Blood Parameters of Broiler Chickens

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Abstract

One-day-old Hubbard broiler chicks were fed basal diet supplemented with bee-pollen at the rate of 0% (control), 0.2, 0.4 or 0.6% for a period of 6 weeks with an aim to use them in broiler nutrition as a natural growth promoting substance. Significant ($p<0.01$) differences in live body weight and body weight gain were found between broiler chicks fed the basal diet and those having bee-pollen in their diets during the experiment period. Broiler receiving 0.6% bee-pollen had the highest significant ($p<0.01$) body weight and body weight gains. Bee-pollen supplementation resulted in less feed intake and improved feed conversion ratio compared to the control group. The relative weight of carcass were significantly ($p<0.01$) higher in chicks fed bee-pollen diets. Chicks fed 0.6% bee-pollen diet were found to have highest relative weights of thymus, bursa and spleen and the highest values of the packed cells volume, hemoglobin concentration, red blood cells, white blood cells, hetrophils and lymphocytes. Serum total protein, albumen and globulin were significantly higher in birds fed on bee-pollen compared with the control group. The concentrations of serum uric acid, creatinine, triglycerides, cholesterol, GOT and GPT in chicks fed 0.2, 0.4 and 0.6% of bee-pollen were found to be lower than the control group. It was concluded that supplementation of bee-pollen to the diets of broiler chicks improved the performance, carcass traits and blood parameters.

Key words: Bee-pollen, blood parameters, broiler, carcass traits, performance

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Over the several last years, use of natural products as substitutes for replacement of antibiotics for improving the performance and immune system in animal life is being encouraged. One of the regarded candidates in natural products is bee-pollen. Bee-pollen is an agglomerate of pollen grains from monoflora or multiflora, which are collected by the bee workers and mixed with nectar and secretion from the hypopharyngeal glands such as β-glycosidase enzymes (Carpes *et al*., 2008). Bee-pollen used as a food supplement since it known as a riche source of carbohydrates, proteins, lipids, vitamins and minerals (Isidorov *et al*., 2009; Taha, 2015), flavonoids (Villanueva *et al*., 2002; Attia *et al*., 2011a) and digestive enzymes added by the bees (Shalmany and Shivazad, 2006; Wang *et al*., 2007). Bee-pollen contains most of the essential nutritional elements needed for the growth and development in humans and animals (Bell *et al*., 1983; Villanueva *et al*., 2002; Capcarova *et al*., 2012; Petruska *et al*., 2012). In fact, some nutritionists stated that human beings could live adequately just by eating bee-pollen (Morais *et al*., 2011). It was claimed that 15 g of the examined Spanish bee-pollen was enough to meet the body requirements of the free amino acids (Nagai *et al*., 2007).

The chemical composition of bee-collected pollen correlate with the plant species from which the pollen was collected (Taha, 2015), the environmental conditions, age and nutritional status of the plants during the flowering period (Herbert and Shimanuki, 1978). Bee-pollen has 13-55% carbohydrates, 10-40% proteins, 1-20% lipids, 3-8% water, 0.5-3% minerals, 0.02-0.1% vitamins, 0.04-3% flavonoids and other compounds such as: Resins and antibiotic substances (Carpes *et al*., 2007; Taha, 2015).

Bee-pollen is promoted as a health food with a wide range of nutritional and therapeutic properties (Yamaguchi *et al*., 2006), triggering beneficial effects to human health and the prevention of prostate problems (Shoskes, 2002), arteriosclerosis (Wojcicki *et al*., 1986), tumors (Zhang *et al*., 1995). It has been reported that bee-pollen accelerates mitotic rate, promotes tissue repair and enhances greater toxic elimination (Morais *et al*., 2011). In addition, it has an anti-microbial, anti-inflammatory, anti-mutagenic (Kacaniova *et al*., 2012; Pascoal *et al*., 2014), an antifungal (Garcia *et al*., 2001; Kacaniova *et al*., 2012), an antioxidant (Morais *et al*., 2011), an anti-allergic (Moita *et al*., 2014), an antiviral, a hypolipidemic, a hypoglycemic and an immunostimulating (Komosinska-Vassev *et al*., 2015). Bee-pollen can also improve the cell immune response, the antibody production speed and reinforce the immunological system (Song *et al*., 2005).

Bee-pollen promotes animal growth, improves animal products quality and security, enhances immunizing function of poultry and protects intestinal tract health (Liu *et al*., 2010). Keeping the above facts in view, the present investigation was carried out to evaluate the effect of bee-pollen supplementation as a natural growth promoting substance.

MATERIALS AND METHODS

A total of 420 one-day-old Hubbard broiler chicks were randomly divided into four equal groups, each group contained three replicates of 35 chicks. The chicks were grown in a separate floor pens with wood shavings and exposed to 24 h constant light. Feed and water were supplied *ad libitum*. The ingredients and chemical composition of the basal diet (starter from 1-21 days of age, grower from 22-35 days of age and finisher from 36-42 days of age) are presented in Table 1. All experimental birds were reared as per the recommendations of the National Research Council (NRC., 1994).

Bee-pollen was obtained from the apiary of the Honeybee Research Section, Plant Protection Research Institute, Sakha, Kafrelsheikh, Egypt. Bee-pollen were homogenized to be a fine powder and packed in polyamide-polyethylene bags and stored at -16°C until use. Treatments were prepared by mixing the bee-pollen into the basal diet at the rate of 0% (control), 0.2% (T_1), 0.4% (T_2) or 0.6% (T_3). Moisture, crude protein, ether

Table 1: Composition and calculated analysis of the experimental diets

Ingredients	Starters	Growers	Finishers
Yellow corn	50.48	58.02	63.61
Soybean meal (44%)	32.55	30.8	26.96
Corn gluten meal (62%)	7.1	2.52	1.23
Soybean oil	6	5.5	4.88
Limestone	1.45	1.3	1.2
Dicalcium phosphate	1.69	1.16	1.42
Salt	0.3	0.3	0.3
Premix*	0.3	0.3	0.3
Dl-Methionine	0.1	0.1	0.1
L. Lysine	0.03	-	-
Total	100	100	100
Calculated analysis			
Crude protein (%)	23.01	20.05	18.03
Metabolizable energy (kcal kg^{-1})	3200	3200	3200
Ether extract (%)	2.4	2.5	2.66
Crude fiber (%)	3.5	3.5	3.3
Calcium (%)	1.03	0.9	0.86
Available phosphorus (%)	0.45	0.35	0.39
Methionine (%)	0.5	0.43	0.399
Lysine (%)	1.11	1	0.9

*Each 3 kg of premix contained: vit. A 12000 IU, vit. D 2200IU, vit. E 10 mg, vit. K3 2000 mg, vit. B1 1000 mg, vit. B2 5000 mg, vit. B6 1500 mg, vit. B12 10 mg, pantothenic acid 10 mg, niacin 30 mg, folic acid 1000 mg, biotin 50 mg, choline chloride 300 mg, manganese 60 mg, zinc 50 mg, copper 10 mg, Iron 30 mg, Iodine 1000 mg, selenium 100 mg, cobalt 100 mg and $CaCo_3$ to 3 g

extract, ash and crude fiber of bee-pollen were determined according to the method of AOAC (1994). Mineral elements were determined according to the method of AOAC (1994) by the atomic absorption spectrophotometer. The amino acids profile was determined according to the method of Duranti and Cerlletti (1979), using a Beckman Amino Acid Analyzer. The fatty acids were analysed using gas chromatography according to Bonvehi and Jorda (1997).

Individual body weight, feed consumption and feed conversion ratio were recorded weekly. Mortality of birds was recorded at the day when it occurred. At six weeks of age, six birds from each treatment were randomly chosen, weighed and then slaughtered. Blood samples were collected at the time of slaughtering from each bird. After complete bleeding and feather removal, carcass, liver, gizzard, heart, spleen, bursa and thymus were weighed and their weight was recorded as percentage of body weight.

Blood samples were divided into two equal parts. The first part of blood was collected with heparin as anticoagulant to be used for estimation of hematological parameters testes such as: Red Blood Cells (RBCs) and White Blood Cells (WBCs) according to Natt and Herrick (1952), Packed Cells Volume (PCV) and heamoglobin (Hb) according to Schalm *et al.* (1975) and Hesser (1960), respectively. Counts of Hetrophils (H), Lymphocytes (L) and H/L ratio were measured according to Shen and Patterson (1983). The second part of each blood sample was centrifuged at 4000 rpm for 15 min to separate blood serum. The obtained serum was kept frozen at -18°C until analysed. Serum samples were used for biochemical analysis such as: Total protein, albumin, cholesterol, triglyceride, uric acid, creatinine, Gamma Pyruvic Transferees (GPT) and Glutamic Oxalocetic Transferees (GOT) were estimated by calorimetric methods using commercial kits supplied by Biomericux (Poains, France). Serum globulin was calculated by the difference between serum total protein and albumin, since the fibrinogen usually comprises a negligible fraction (Sturkie, 1976).

Statistical analysis: Data were subjected to the one-way analysis of variance (ANOVA) using SAS® software computer program (SAS., 2003). Treatment means were compared using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS

Bee-pollen composition: Bee-pollen has 62.82% carbohydrates, 19.23% proteins, 4.09% lipids, 2.28% ash and 0.90% Fiber. The most prevalent amino acids in bee-pollen were glutamic acid, glycine, aspartic acid, alanine, leucine, valine and lysine. Palmitic (C16:0), linolenic (C18:3), oleic (C18:1) and linoleic (C18:2) were the most predominant fatty acids (Table 2).

Body weight or body weight gain: The live body weight at one day of age for all treatments was nearly similar. It was found that, there were significant ($p<0.01$) differences in average values of either live body weight or body weight gain between broiler chicks fed basal diet and those having bee-pollen in their diets at all experimental periods. At the end of the experimental period, the highest values (g) of live body weight (2312.33) or body weight gain (2270.91) were obtained from chicks fed diet contained 0.6% bee-pollen (Table 3).

Table 2: Chemical composition of bee-pollen

Chemical analysis	%
Dry matter	90.32
Lipids	4.09
Protein	19.23
Carbohydrate	62.82
Ash	3.28
Fibers	0.90
Amino acids (g/100 g protein)	
Arginine	2.11
Histidine	2.79
Iso-leucine	3.49
Leucine	6.67
Lysine	4.49
Methionine	0.28
Phenylalanine	1.46
Threonine	2.72
Valine	5.32
Alanine	7.23
Aspartic acid	9.31
Glutamic acid	9.67
Glycine	9.44
Proline	0.32
Serine	4.53
Tyrosine	1.11
Fatty acids (%)	
Palmitic ($C_{16:0}$)	19.36
Stearic ($C_{18:0}$)	5.55
Oleic ($C_{18:1}$)	14.20
Linoleic ($C_{18:2}$)	10.14
Linolenic ($C_{18:3}$)	16.64
Arachidic ($C_{20:0}$)	3.27
Behenic ($C_{22:0}$)	6.39
Minerals (mg kg^{-1})	
Na	381.50
K	5030.46
Ca	3496.56
Mg	1375.64
P	328.53
Fe	121.50
Mn	21.99
Zn	36.14
Cu	6.47

Table 3: Effect of different levels of bee-pollen on average weekly live body weight (g) and body weight gain (g) of broilers chicks

	Periods (weeks)														
	Live body weight gain (g)						Live body weight (g)								
Treatments	1 day	1	2	3	4	5	6	0-1	1-2	2-3	3-4	4-5	5-6	0-6
Control	41.53±0.2	178.04±0.08^{d}	400.52±0.17^{d}	700.00±0.20^{d}	1211.82±0.1^{d}	1711.82±0.19^{d}	2075.46±0.11^{d}	136.51±0.54^{c}	222.48±0.09^{d}	299.48±0.14^{b}	511.82±0.18^{d}	500.0±0.17^{b}	363.64±0.30^{c}	2033.93±0.24^{d}
T_1 (0.2%)	41.48±0.23	178.73±0.11^{c}	410.32±0.24^{c}	734.29±0.13^{c}	1250.48±0.18^{c}	1787.62±0.21^{c}	2230.95±0.40^{c}	137.25±0.06^{c}	231.59±0.02^{c}	323.97±0.31^{a}	516.19±0.31^{c}	537.14±0.30^{c}	443.33±0.13^{b}	2199.47±0.32^{c}
T_2 (0.4%)	41.37±0.20	181.15±0.13^{b}	420.33±0.20^{b}	748.50±0.20^{b}	1268.42±0.23^{b}	1807.65±0.08^{b}	2255.56±0.20^{b}	139.78±0.09^{b}	239.18±0.30^{b}	328.17±0.08^{a}	519.92±0.17^{b}	539.23±0.24^{b}	447.91±0.24^{a}	2214.19±0.30^{b}
T_3 (0.6%)	41.42 ±0.18	184.20±0.17^{a}	426.25±0.03^{a}	754.74±0.13^{a}	1282.00±0.20^{a}	1864.20±0.19^{a}	2312.33±0.21^{a}	142.78±0.1^{a}	242.05±0.11^{a}	328.49±0.021^{a}	527.26±0.24^{a}	582.2±0.19^{a}	448.13±0.31^{a}	2270.91±0.32^{a}
Significance	NS	**	**	**	**	**	**	**	**	**	**	**	**	**

Means of each column followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test, ** and NS indicate p<0.01 and not significant, respectively

Table 4: Effect of different levels of bee-pollen on feed consumption (g) and Feed conversion ratio of broiler chicks

	Periods (weeks)													
	Feed consumption (g/bird/period)							Feed conversion ratio						
Treatments	0-1	1-2	2-3	3-4	4-5	5-6	0-6	0-1	1-2	2-3	3-4	4-5	5-6	0-6
Control	155.23±0.19^{a}	375.63±0.14^{a}	476.19±0.3^{a}	845.82±0.21^{a}	949.71±0.19^{a}	1090.91±0.26^{a}	3893.49±0.11^{a}	1.14±0.18	1.69±0.06^{a}	1.59±0.08^{a}	1.65±0.11	1.90±0.02	2.99±0.07^{a}	1.911±0.08^{a}
T_1 (0.2%)	145.92±0.2^{d}	365.63±0.41^{d}	446.36±0.13^{d}	837.28±0.11^{d}	934.55±0.36^{d}	1039.68±0.25^{d}	3769.42±0.01^{d}	1.03±0.08	1.58±0.02^{ab}	1.38±0.01^{b}	1.62±0.03	1.74±0.03	2.35±0.03^{b}	1.710±0.02^{b}
T_2 (0.4%)	149.55±0.14^{c}	369.36±0.20^{c}	448.44±0.21^{c}	838.26±0.24^{c}	936.43±0.08^{c}	1047.83±0.21^{c}	3789.87±0.01^{c}	1.07±0.02	1.54±0.17^{b}	1.37±0.02^{b}	1.61±0.01	1.74±0.17	2.34±0.03^{b}	1.710±0.05^{b}
T_3 (0.6%)	150.45±0.357^{b}	371.81±0.35^{b}	450.45±0.07^{b}	842.85±0.19^{b}	947.45±0.18^{b}	1049.90±0.17^{b}	3812.91±0.01^{b}	1.05±0.03	1.54±0.02^{b}	1.37±0.02^{b}	1.60±0.02	1.63±0.01	2.34±0.04^{b}	1.680±0.02^{b}
Significance	**	**	NS	NS	**	*	NS	**	NS	*	NS	NS	**	**

Means of each column followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test, ***and NS indicate p<0.01, 0.05 and not significant, respectively

Feed consumption and conversion: The feed consumption was decreased significantly (p<0.01) in birds received bee-pollen diets compared with the control group during the all experimental periods. Birds in control group consumed feed more than birds fed on bee-pollen (0.2, 0.4 and 0.6%) in their diets (3893.49 g bird^{-1} vs. 3769.42, 3789.87 and 3812.91 g bird^{-1}), respectively (Table 4). Feed conversion was improved in parallel with the increasing of the bee-pollen supplementation level.

Carcass: The relative weight of carcass increased significantly (p<0.01) in groups fed bee-pollen diets compared to the control group. The highest values (%) of relative weight of gizzard (2.21) and liver (2.07) were obtained from chicks fed on the basal diet while the highest values (%) of relative weight of heart (0.47), thymus (0.40), bursa (0.11) and spleen (0.15) were obtained from chicks fed on bee-pollen at 0.6% level (Table 5).

Hematological parameters and blood constituents: The hematological parameters and blood constituents of broilers chicks were affected significantly by dietary bee-pollen supplementation (Table 6 and 7). The values of PCV, Hb, RBCs, WBCs, H and L in chicks fed on the basal diet were lower than that of chicks fed bee-pollen at any level in their diets while the highest value of H/L was obtained in birds fed control diet. The highest values (g/100 mL) of total protein (5.30), albumin (2.90), globulin (2.40) and the lowest serum uric acid (2.18), creatinine (0.70), triglycerides (176.90), cholesterol (196.00), SGOT (63.0 UL^{-1}) and SGPT (67.30 UL^{-1}) levels were obtained from chicks fed diet supplemented with 0.6% bee-pollen.

Mortality: The mortality percentage (2.16%) of broiler chicks fed different levels of bee-pollen during the whole experimental period was almost similar the control group, therefore results were not tabulated.

DISCUSSION

Broiler receiving 0.6% bee-pollen (T_3) in their diet had significantly (p<0.01) highest body weight and body weight gains during the experimental periods. The birds received 0.2, 0.4 or 0.6% bee-pollen showed an increasing in body weight gain by 8.14, 8.86 and 11.65% compared to the control birds, respectively. These positive improvements could be due to the nutritive value of the bee-pollen as a rich source of protein (19.32%), essential amino acids (e.g., Leucine and lysine), fat (4.09%), unsaturated fatty acids (e.g., oleic, linoleic and

Table 5: Effect of different levels of bee-pollen on relative weight of carcass and organs

Treatments	Carcass	Gizzard	Liver	Heart	Thymus	Bursa	Spleen
Control	73.11±0.11^{d}	2.21±0.02	2.07±0.06	0.46±0.01^{a}	0.28±0.01^{c}	0.05±0.03^{b}	0.11±0.01
T_1 (0.2%)	75.56±0.08^{c}	2.21±0.03	2.01±0.06	0.39±0.02^{b}	0.32±0.04^{b}	0.06±0.01^{b}	0.12±0.05
T_2 (0.4%)	76.75±0.09^{b}	2.19±0.05	2.02±0.07	0.46±0.01^{a}	0.39±0.01^{a}	0.07±0.02^{b}	0.14±0.03
T_3 (0.6%)	78.50±0.08^{a}	2.19±0.03	2.03±0.05	0.47±0.03^{a}	0.40±0.01^{a}	0.11±0.02^{a}	0.15±0.02
Significance	**	NS	NS	*	**	**	NS

Means of each column followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test. **, * and NS indicate p<0.01, 0.05 and not significant, respectively

Table 6: Effect of different levels of bee-pollen on hematological parameters of broilers chicks at 6 weeks of age

Treatments	PCV (%)	Hb (g/100 mL)	RBC (×10^{6}/100 mL)	WBC (×10^{3}/100 mL)	H (10^{3}/100 mL)	L (10^{3}/100 mL)	H/L ratio
Control	27.00±0.1^{d}	9.50±0.04^{b}	1.40±0.06^{b}	22.01±0.05^{d}	20.67±0.38^{c}	34.67±0.44^{c}	0.62±0.01
T_1 (0.2%)	30.00±0.03^{c}	10.00±0.06^{b}	1.55±0.05^{a}	23.04±0.04^{c}	21.25±0.02^{bc}	35.25±0.11^{bc}	0.60±0.05
T_2 (0.4%)	32.50±0.02^{b}	11.50±0.02^{a}	1.60±0.04^{a}	24.01±0.01^{b}	21.66±0.01^{b}	36.00±0.01^{b}	0.60±0.01
T_3 (0.6%)	34.30±0.05^{a}	12.00±0.05^{a}	1.64±0.01^{a}	24.50±0.07^{a}	22.50±0.11^{a}	36.90±0.11^{a}	0.61±0.2
Significance	**	**	**	**	**	**	NS

PVC: Packed cell volume, Hb: Heamoglobin, RBC: Red blood cells, WBC: White blood cells, H: Hetrophils, L: Lymphocytes and H/L: Hetrophils ratio lymphocytes. Means of each column followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test. ** and NS indicate p<0.01 and not significant, respectively

linolenic), carbohydrates (62.82%) and minerals (e.g., Na, K, Ca, Mg, P, Zn, Mn, Fe and Cu) (Table 2). Also bee-pollen increased the intestinal absorptive capacity through the longer and thicker villi and it stimulates the digestive and absorptive functions of broilers (Wang *et al*., 2007) in association with a significant improvement in body weight gain due to higher protein anabolism (Attia *et al*., 2011a) and bee-pollen has many of enzyme which support the digestive system (Hascik *et al*., 2012). These results are in agreement with the findings of Wang *et al*. (2007), Han *et al*. (2010), Hascik *et al*. (2012), Attia *et al*. (2014a) and Coskun *et al*. (2014) for broiler chicks and El-Hanoun *et al*. (2007), Shewika (2009), Attia *et al*. (2011b), El-Neney and El-Kholy (2014) for rabbit, Wang and Cheng (2005) for pigs, Zhang *et al*. (2010) for calves and Abbass *et al*. (2012) for *Nile tilapia*.

In comparison to the control group, feed consumption was decreased significantly (p<0.01) in chicks supplemented with bee-pollen diets during the all experimental periods. At all investigated periods, birds received bee-pollen (0.2, 0.4 or 0.6%) showed an improvement in feed conversion ratio compared to the control group. The best feed conversion ratio was obtained in the 0.6% bee-pollen supplementation (T_3) for the entire experimental period. The current results are in contrast with those observed by Attia *et al*. (2014a) and Abou El-Naga (2014) for broiler chicks, Hedia *et al*. (2007), Attia *et al*. (2011a) and Attia *et al*. (2014b) for rabbit, Wang and Cheng (2005) for pigs, Zhang *et al*. (2010) for calves and Abbass *et al*. (2012) for fish. The improvement of feed conversion and lower feed intake in the present study might be due to the vitamins, amino acids, hormones and minerals contained in bee-pollen, as well as enzymes and coenzymes added by bees during formation of pellets, which are necessary for good digestion and cell growth (Wang *et al*., 2007). On contrary, Han *et al*. (2010) and Hascik *et al*. (2012) reported that bee-pollen increased feed intake of broiler chickens.

In comparison to the control group, feeding broiler chicks on bee-pollen diets (0.2, 0.4 and 0.6%) showed an increase in the relative weight of carcass. The increase in the weight of carcass might be due to the increasing in the growth performance and digestibility. The relative weight of gizzard and liver were insignificantly decreased in broiler received bee-pollen compared with the control. There were no significant differences in the relative weight of heart between the broilers that fed 0.4 or 0.6% bee-pollen while it was significantly (p<0.05) decreased in the birds received 0.2% bee-pollen compared to the control group. These results are agreed with Hascik *et al*. (2012) who found that the weights of carcass and giblets were insignificantly increased in the male chickens fed bee-pollen (400 mg kg^{-1}) compared with the control group. In addition, Attia *et al*. (2014a) reported that the supplementation of bee-pollen in diets of broiler increased the dressing percentage while the percentage of the gizzard, liver and heart were un-affected compared with the control treatment. El-Neney and El-Kholy (2014) found that the addition of bee-pollen to diets of rabbits increased the carcass traits. On the other hand, Song *et al*. (2005) and Wang *et al*. (2007) noticed that the liver and pancreas weights of broilers fed with bee-pollen were higher than those weights of the control group. However, addition of bee-pollen did not influence the carcass yield of the birds (Ke *et al*., 2009) and the rabbits (Dias *et al*., 2013; Attia *et al*., 2014b).

The relative weights of lymphoid organs were significantly increased in broiler received diet contained bee-pollen, except of spleen weight that was insignificantly increased. In general the highest relative weights of

Table 7: Effect of different levels of bee-pollen on blood constituents of broilers chicks at 6 weeks of age

Treatments	Total protein (g/100 mL)	Albumin (g/100 mL)	Globulin (g/100 mL)	Uric acid (g/100 mL)	Creatinine (g/100 mL)	Triglycerides (g/100 mL)	Cholesterol (g/100 mL)	SGOT (UL^{-1})	SGPT (UL^{-1})
Control	4.40±0.06^{d}	2.50±0.6^{c}	1.90±0.04^{d}	4.00±0.20^{a}	1.20±0.03^{a}	182.00±0.17^{a}	208.00±0.6^{a}	67.00±0.08^{a}	71.10±0.22^{a}
T_1 (0.2%)	4.60±0.22^{c}	2.60±0.01^{b}	2.00±0.11^{c}	3.09±0.10^{b}	1.10±0.01^{a}	179.00±0.27^{b}	199.00±0.07^{b}	65.00±0.07^{b}	69.00±0.06^{b}
T_2 (0.4%)	4.90±0.16^{b}	2.60±0.02^{b}	2.30±0.06^{b}	3.12±0.14^{b}	1.10±0.57^{a}	178.00±0.41^{c}	198.00±0.57^{b}	64.00±0.3^{bc}	69.20±0.16^{b}
T_3 (0.6%)	5.30±0.33^{a}	2.90±0.56^{a}	2.40±0.01^{a}	2.18±0.21^{c}	0.70±0.06^{b}	176.90±0.05^{d}	196.00±0.04^{c}	63.00±0.13^{c}	67.30±0.33^{c}
Significance	**	**	**	**	**	**	**	*	*

Means of each column followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test. **, * and indicate p<0.01 and 0.05, respectively

thymus, bursa and spleen were found in the birds gave 0.6% bee-pollen. These results indicated that supplement bee-pollen in chicks diet promoted the growth of thymus, bursa and spleen that resulted in positively influence the immunity of broiler. Bee-pollen can also improve the cell immune response, the antibody production speed and reinforce the immunological system (Song *et al*., 2005). Bee-pollen enhanced lymphocytes percentage which leads to produce more T-cells in rabbit (El-Neney and El-Kholy, 2014). T-cells undergo maturation in the thymus gland and play a major role in cell-mediated immunity (Stephen, 2007). These positive effects may be due to the bee-pollen which has nucleic acids that stimulate the natural killer cells and T-lymphocyte activities such as: Vitamin A and E and microelements such as Fe, Se, Zn and Mn, which promote the proliferation and differentiation of immune system cells (Wang *et al.,* 2005). These results are in agreement with Wang *et al*. (2005), who reported that the absolute weight of spleen increased in broiler fed 1.5% bee-pollen. Also bee-pollen could boost the early development of thymus and bursa, retard the bursa degeneration and promote the immune response of spleen in chickens. The spleen weight of broiler feed diets contained bee-pollen (0.5, 1 and 1.5%) was higher from the 7th up to 42nd day of age (Cheng, 2009). In another experiment, Dias *et al*. (2013), Attia *et al*. (2014b) and El-Neney and El-Kholy (2014) noted that spleen percentage was higher in rabbits treated with bee-pollen.

The Packed Cells Volume (PCV), Hemoglobin Concentration (Hb) and Red Blood Cells (RBCs) in birds fed bee-pollen diet (0.2, 0.4 or 0.6%) were significantly higher than these values of the control group. Bee-pollen was found to cause increases in hemoglobin and serum iron (Xie *et al.,* 1994), the RBC count, Hb and PCV values (Attia *et al.,* 2014a) in broiler chickens, hemoglobin, red blood cells count and hematocrite in rabbits (El-Neney and El-Kholy, 2014) and the number of red blood cells in human (Iversen *et al.,* 1997). The highest value of them were detected in chicks fed 0.6% bee-pollen. These results indicated that feeding on bee-pollen increased Hb and RBCs lead to help increase energy levels. Since the hemoglobin in red blood cells that carry oxygen for energy metabolism, this may explain the relationship between bee-pollen and energy. The positive impact on PCV and Hb and RBCs values could be due to minerals such as Fe and Cu contained in bee-pollen (Table 2) and vitamins such as folic acid and vitamin C. These minerals and vitamins have a role in red blood cell formation and maturation (El-Wafa *et al*., 2002) which in turn caused an increase in hemoglobin and hematocrite, so there are positive relationships between RBC's and hemoglobin and hematocrite (Sturkie, 1986).

White Blood Cells (WBCs) were found to be significantly increased in birds fed bee-pollen diet contained 0.2, 0.4 or 0.6% when compared with the control group. The increasing of white blood cells in a certain level is a good indicator of increasing the immunity efficiency. This result confirmed by the findings of Attia *et al.* (2014a) for broiler chicks and Hedia *et al*. (2007), Attia *et al*. (2014b) and El-Neney and El-Kholy (2014) for rabbits.

Hetrophils (H) and Lymphocytes (L) were increased significantly (p<0.01) in broiler fed 0.4 and 0.6% bee-pollen compared to the control group. The lymphocyte is considered the main type of white blood corpuscles and a good indicator of the increase in immune efficiency (Swiderek *et al*., 2006). Bee-pollen can enhance immunity (Song *et al*., 2005; Liu *et al*., 2010; Attia *et al*., 2011a, b; Popiela-Pleban *et al*., 2012). The improvements in the immune response could be due to the presence of minerals, antioxidant which present in flavonoids and vitamins in bee-pollen which have a role in enhancing immune system. The relationship between antioxidant and immune cells may be explained with the fact that the immune cell function is linked to the release of Reactive Oxygen Species (ROS) even though this ROS is involved in the microbicidal and cytotxic activity of immune cells, excessive amount of it is harmful for immune cells as it attack cellular components and lead to cell damage or death. This ROS can be scavenged by antioxidants leading to improve immune cell functions (De la Fuente and Victor, 2000; Victor *et al*., 2003; Hughes, 1999; Barillari *et al*., 2005). Antioxidants are very important to immune defense and animal health, consequently, to their production capacity (Chew, 1995). Natural antioxidant was found to stimulate cell-mediated

immunity of rabbits (El-Kholy *et al*., 2008). Similar results were obtained by El-Neney and El-Kholy (2014) who noticed significant increase in lymphocytes type in rabbit received 200, 300 and 400 mg bee-pollen/kg body weight daily compared to the control.

Broiler fed diets containing bee-pollen (0.2, 0.4 or 0.6%) had insignificantly lower H/L ratio than those fed the control diet. The H/L ratio was found to be associated with increased levels of stress in the bird (Vijayan and Rema, 1997). The H/L ratio is a good measure of the chicken's perception in its environment and increasing H/L ratio indicated that the birds were under acute stress (Al-Daraji *et al.,* 2002). The H/L ratio has been found as general biomarker relevant to immune function in poultry (Shini, 2003).

Serum biochemical parameters have been used as indicators of the nutritional and physiological status of birds. Serum total protein, albumen and globulin increased significantly ($p<0.01$) in birds received 0.2, 0.4 or 0.6% bee pollen compared with the control group. The highest values of them were recorded in chicks fed diets containing 0.6% bee-pollen. The increase in the plasma protein and albumin may be due to the high concentrations of protein and amino acids contained bee-pollen (Bell *et al*., 1983). Also bee-pollen improved liver function. It is well known that the changing in the plasma albumin level reflects the change in liver function, where the liver is the site of albumin synthesis while the globulin formed by lymphatic tissues (Jones and Bark, 1979). These results are in agreement with the findings of Attia *et al.* (2014a), who noted that bee pollen has a positive effect on serum total protein, albumen and globulin in broiler chicks. Also, Hedia *et al*. (2007), Attia *et al*. (2011a, b) and El-Neney and El-Kholy (2014) observed the same trend in rabbit.

Serum uric acid level showed significant ($p<0.001$) decrease in broiler received 0.2, 0.4 or 0.6% bee-pollen compared with the control. The levels of creatinine were similar in broiler received 0.2, 0.4% bee-pollen and control diet while birds fed diet containing 0.6% bee-pollen recorded significantly decreased in the creatinine level. These results showed that the bee-pollen dietary improved kidney health in terms of filtration rate. Significant increases in serum uric acid and creatinine levels are indicative of nephrotoxicity in broiler chickens (Huff *et al*., 1988). These results are agreement with the findings of Nagyova *et al.* (1994), Hu *et al*. (2003) and Attia *et al*. (2014a), who reported that bee-pollen reduced urea-N and creatinine concentrations in broiler chickens fed diet supplemented with bee-pollen compared with the control treatment. Also, Hedia *et al*. (2007), Attia *et al*. (2011a, b, 2014b) and El-Neney and El-Kholy (2014) found the same results in rabbit.

The results showed that there was a significantly ($p<0.001$) decrease in average values for serum triglycerides and cholesterol levels with increasing the duration of adding bee-pollen to the broiler diet. The decreasing in serum triglycerides and cholesterol values may be due to unsaturated acids; oleic, linoleic and linolenic (14.20,10.14 and 16.64% of fatty acids, respectively) in bee-pollen (Table 2) that inhibits accumulation of lipid peroxidation product. The decrease in plasma lipids and cholesterol could be due to phospholipids and PUFA particularly linolenic fatty acid which represented by 1.19% in bee-pollen (Xu *et al*., 2009). The dietary monounsaturated fatty acids (e.g., oleic acid) were very effective in lowering blood cholesterol concentration and may be important in preventing coronary heart disease (Hornstra, 1980). Data obtained herein are in agreement with those obtained by Attia *et al.* (2014a), who noticed that bee-pollen can decrease the level of triglycerides and cholesterol in broiler chicks. Also, Hu *et al*. (2003) found the same results in rats.

Concerning liver function, the Serum Glutamic Oxaloacetic Transaminase (SGOT) and the Serum Glutamic Pyruvic Transaminase (SGPT) decreased significantly ($p<0.001$) in birds fed bee-pollen diets (0.2, 0.4 or 0.6 %) compared to the control group. These findings suggested that bee-pollen improved liver function and reduced liver damage. The protective effect of bee-pollen upon liver could be due to antioxidant contents of some flavonoids such as quercerin and rutin which play a role as antioxidant against oxidative material which caused damage to liver (El-Neney and El-Kholy, 2014). According to Attia *et al*. (2014a) the level of SGOT was reduced because of the administration of bee-pollen in broiler's diet. On other animals, Wojcicki *et al*. (1985) found that the protective effect of pollen extracts against allyl alcohol damage of the liver in mice reduced SGOT, SGPT and alkaline phosphatase activities. Supplementation of fish diet with honey bee-pollen (2.5%) decreased the serum GPT level, contrary to the serum GOT level when compared to the control group (Abbass *et al*., 2012). Also, Hedia *et al*. (2007), Attia *et al*. (2011a, b, 2014b) and El-Neney and El-Kholy (2014) found that supplementation of bee-pollen to the diet of the rabbit caused significant decrease in serum GOT and GPT compared to the control group.

CONCLUSION

It was concluded that bee-pollen is confirmed as an interesting resource, able to improve performance, carcass traits, immunity and blood parameters of broiler chicks. Standing on the present results, the best concentration of

bee-pollen in broiler diets is 0.6% during starter, grower and finisher periods.

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