



Effect of High Density of Birds' numbers and Bee Pollen Addition on The level of Heat Shock Protein 90 and Blood Parameters of Male Japanese Quails



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THE aim of this study is to determine the role of bee pollen in improving the blood parameters and heat shock proteins 90 of male quail exposure to high floor density. The total number was (300) 7-week-old birds, distributed randomly into 6 experimental groups, for 8 weeks. The 1st group was put of 21 birds / m², the 2nd group was put of 21 birds / m² and bee pollen (BP) was added at a 20 g/kg feed, the 3rd group was put of 21 birds / m² and added of BP at dose 30 g/kg feed. The 4th group was high density (HD) 75 birds/m², the 5th group was HD 75 birds/m² and supplemented with BP at a 20 g/kg feed, and the 6th group was HD 75 birds/m² and supplemented with BP at dose 30 g/kg feed. The results showed HD for birds caused a significant decrease in hemoglobin concentration, packed cell volume, mean corpuscle hemoglobin concentration (MCHC), lymphocyte number, albumin level, glutathione, and a significant increase in stress index, Heterophil number, heat shock protein 90 (HSP), and alanine aminotransferase (ALT). The addition of 20 and 30 BP to HD birds resulted in a significant increase in hemoglobin concentration, lymphocyte number, and albumin, while causing a significant decrease in stress index, heterophil cells number, HSP, and malondialdehyde (MDA). It is concluded that adding BP alone did not affect the normal parameters of blood values, while adding it to quail birds that suffer from high population density improved blood parameters and reduced stress.

Keywords: Bee Pollen, Blood parameters, High density quail's numbers and Heat shock protein 90.

Introduction

Poultry breeders work to increase the density of bird numbers as an administrative practice used to reduce the cost associated with labor and the lack of space inside poultry breeding halls as well as the lack of equipment or tools used inside the breeding halls [1]. Poultry breeders often increase the density of birds per unit area, but this always leads to increased heat and thus leads to heat stress as the higher density of birds contributes to poor bird performance [2]. It thus increases heat shock proteins in the muscles and heart, which may enhance stress and increase the survival rate of stressed cells [3]. Hematological

biochemical tests of blood are mainly used as indicators of the physiological and nutritional status of broiler chickens [4]. Some authors [5] showed a significant correlation coefficient between stress and a decrease in the number of white blood cells. Exposure to stress leads to an increase in the concentration of corticosterone in poultry species, including quail, where high levels of corticosterone in the blood lead to an increase in the Heterophil/lymphocyte ratio [6]. It was proved that increasing the number of birds per unit area causes oxidative stress in broilers as it increases the level of malondialdehyde and reduces the activity of glutathione peroxidase in the blood serum [7]. In recent years, the

importance of feed additives has increased in the prevention and treatment of diseases, as natural additives such as bee products, probiotics, and plant extracts have been used because of the physiological benefits of these materials to the body and reduce the risk of diseases as well as the positive effects of the immune system, where bee pollen attracted the attention of researchers because it contains phenols and flavonoids [8]. Bee pollen is a mixture of flower pollen accumulated by nectar and salivary enzymes of honey bee workers. The main components of bee pollen are proteins, essential amino acids, reducing sugars, lipids, nucleic acids, minerals, and vitamins, as well as enzymes and coenzymes required for good digestion [9]. Abdelnour et al. [10] found that nutritional bee pollen improved blood picture and biochemistry, as well as heart, liver, and kidney functions. Confirmed by Wan et al. [11] that bee pollen significantly increased the activity of glutathione in the blood serum, lowered the level of MDA, and improved the antioxidant capacity in broiler chickens. The study's goal is to investigate the effect of bird population density as well as the role of bee pollen in blood constituent, liver enzymes, glutathione, malondialdehyde, total protein, stress index, and heat shock protein.

Material and Methods

Ethical approval:

All techniques used in the experiment were approved by the College of Veterinary Medicine /University of Mosul, Animal Ethics Committee (Iraq).

Quail birds' males

This research was conducted in the animal house located in the College of Veterinary Medicine at the University of Mosul. Three hundred (300), 7-week-old Japanese quail were taken from the Agricultural Research Department of the Nineveh Agriculture Directorate. The quail were fed on the basic diet [12]. With drinking

water provided for the duration of the 8-week experiment, the illumination was constant for 14 hours a day, and the room temperature ranged from 20 °C to 25 °C. The relative humidity ranged from 45 to 60%.

Experimental design

Male quails were randomly distributed to 6 experimental groups, three of which (21 birds) were divided into three replicates, with 7 birds for each replicate (3 x 7), and three other groups (75 birds) were also divided into three replicates, with 25 birds for each replicate. (3 x 25). 1st group at a rate of 21 birds/m² (control), at normal density (ND), and fed the basic diet. The 2nd group at a rate of 21 birds/m² and the addition of diet with bee pollen 20 g/kg of feed, the 3rd group at a rate of 21 birds/m² and fed diet with the addition of BP 30 g/kg of feed, the 4th group of high density (HD) of 75 birds/m² on basal feed, 5th group high-density (HD) 75 bird/m² and diet supplemented with BP 20 g/kg feed, 6th group high-density (HD) 75 bird/m² and diet supplemented with BP 30 g/kg feed.

Materials

Basal Ration

The birds were fed from the productive ration from the Andalus lab, according to the National Research Council [12].

Bee pollen and dose

The birds were fed from the productive ration with the addition of bee pollen obtained from the local markets of the city of Mosul, and it was collected by bees from palm trees in the city of Hilla. It was given in two doses of 20 g/kg and 30 g/kg mixed with the ration and according to the groups.

Studied parameters

Weights of internal organs

Liver weight was calculated as organ weight/100 g of body weight [13].

The ingredients	%
Wheat	22
Crushed yellow corn	42
Animal protein	4
Vegetable oil	1
Soybean	30
Salt	0.3
Limestone	0.7

Blood parameters tests

Non clouted blood samples were collected from the jugular vein at the end of the experiment for 36 birds, 6 birds for each group. In Test tubes containing EDTA anticoagulant for of blood tests. (Hb) concentration, according to the Drabkin method [14], packed cell volume, mean corpuscular (Hb) concentration, differential leucocyte count using Wright stain [15], and stress index [16]. Test tubes containing gel were used, and the blood samples were left to clot at room temperature, then serum was separated. The blood was centrifuged for 15 minutes at 3000 rpm and stored in sealed plastic tubes (Eppendorf tubes) and kept at -20 °C for the purpose of performing laboratory serological tests.

Blood biochemical and Liver function tests

Glutathione level estimation, it was determined using methods of Burtis and Ashwood [17]. Malondialdehyde level estimation, the level of lipid peroxidation in the blood serum was determined by measuring the amount of malondialdehyde [18]. Heat-shock protein determination, a ready-made assay kit from Sun Long Biotech was used with ELISA technology. Determination of the level of alanine aminotransferase and aspartate aminotransferase. ALT and AST estimation kit from Biolabo Company was used. Determination kits from Biolabo Company was used for the estimation of total protein, albumin, and globulin.

Statistical analysis

The data was statistically analyzed using one-way analysis of variance, with significant differences between groups tested using Duncan's multiple range test at a probability level ($P < 0.05$).

Result

The results in Table 1 demonstrated that adding 20 g and 30 g of BP/kg diet to a group of birds 21 birds/m² in comparison to the control group (number of birds 21 birds/m²) did not significantly alter the concentration of hemoglobin, the percentage of the packed cell volume, the mean corpuscle hemoglobin concentration, or the stress index. The high bird density (75 birds/m²) resulted in a significant decrease ($P \leq 0.05$) in Hb, PCV, and MCHC and an increase in the stress index when compared to the control group. The high density of birds 75 birds/m² caused a significant decrease ($P \leq 0.05$) in Hb, PCV and MCHC, accompanied by a rise in the stress index Compared with the control group. The results of the study indicated that adding 20 g and 30 g of bee pollen/kg diet to the group density of birds 75 birds/m² significantly increased ($P \leq 0.05$) in hemoglobin concentration accompanied by a decrease in the stress index compared with the group density of birds (75birds/m²) that were not given bee pollen. The addition of 30 g of bee pollen to the density group of 75 birds/m² resulted in a significant increase ($P \leq 0.05$) in the MCHC compared with the density group that was not given bee pollen.

TABLE 1. Effect of high density of bird's numbers and the addition of bee pollen to the diet on the blood parameters of quail males

Treatment	Parameters(Mean ± SE)			
	Hemoglobin concentration g/100ml	Packed cell volume (PCV) %	Mean corpuscle hemoglobin concentration	Stress index
(G1) Control group (n=21 birds)	19.42 ± 0.45 ^a	40.16 ± 0.79 ^b	48.33 ± 0.33 ^a	0.81 ± 0.06 ^b
(G2) Birds numbers 21 , and add 20g/kg pollen	19.80 ± 0.94 ^a	43.83 ± 1.88 ^{bc}	46.39± 1.13 ^{ab}	0.63 ± 0.08 ^{bc}
(G3) Birds numbers 21 and add 30g/kg pollen	19.05±0.59 ^a	44.83 ± 2.15 ^b	45.16 ± 0.79 ^{ab}	0.88 ± 0.08 ^b
(G4) High density group (n=75 birds/m ²)	16.39 ± 0.61 ^b	34.33 ± 0.71 ^c	42.74± 1.19 ^c	1.29 ± 0.16 ^a
(G5) High density group (n=75 birds/m ²) and add 20g/kg pollen	18.99 ± 0.40 ^a	41.00 ± 0.73 ^{bc}	44.63 ± 1.14 ^{bc}	0.45 ± 0.02 ^c
(G6) High density group (n=75 birds/m ²) and add 30g/kg pollen	18.83 ± 0.59 ^a	42.33 ± 1.68 ^{bc}	47.66± 0.93 ^{ab}	0.68 ± 0.07 ^{bc}

Values using different letters in the column are significantly different $P < 0.05$.

The statistical analysis results in Table 2 did not show a significant difference ($P>0.05$) in the percentage number of lymphocytes and heterophils in the group of 21 birds / m² with 20 and 30 gm of bee pollen / kg diet compared to the control group of birds. The density of the number of birds (75 birds/m²) resulted in a significant decrease ($P\leq 0.05$) in the number of lymphocytes and a significant increase in the percentage of heterophils compared with the values of the control group. Addition of 20 and 30 g of bee pollen to the diet of the density group of birds (75 birds/m²) caused a significant increase ($P\leq 0.05$) in the number of lymphocytes accompanied by a significant decrease in the number of heterophils compared with the density group of 75 birds that were not given bee pollen. The data of the results of the study entered into the statistical program did not show any significant difference ($P>0.05$) in the percentage of numbers of eosinophil, basophil and monocyte among the groups.

It was not observed from Table (3) any significant differences ($P>0.05$) in the level of total proteins and globulin when comparing between groups. And there was no significant difference ($P>0.05$) in the level of albumin when comparing the two groups of birds with 21 birds/m² and adding to their diet 20 and 30 gm of bee pollen compared with the control group (21 birds/m²). The high density of birds (75 birds/m²) caused

a significant decrease ($P\leq 0.05$) in the albumin level compared with the control group (21 birds/m²). Adding bee pollen at a dose of 20 and 30 g/kg ration to the group density of birds 75 birds/m² led to a significant increase ($P\leq 0.05$) in the albumin level compared with the group density of birds at 75 birds/m² that were not given bee pollen.

In comparison to the control group, neither the group of birds prepared at 21/m² and fed 20 g/kg of bee pollen nor the group of birds at 21/m² and fed 30 g/kg of bee pollen significantly differed in the concentration of heat shock proteins 90, the level of malondialdehyde, or the level of glutathione.

The current findings revealed that, when compared to the control group (bird numbers 21 birds/m²), the concentration of heat shock proteins 90 and the level of malondialdehyde significantly increased, while the level of glutathione significantly decreased. The results for adding 20 and 30 g/kg of bee pollen to the group of birds numbering 75 birds /m² was a significant decrease in the level of heat shock proteins 90 and in the level of malondialdehyde, accompanied by a significant increase in the level of glutathione, except for the group density of birds at 75 birds / m² and the addition of bee pollen 30 g, which did not show a significant difference in the level of malondialdehyde compared with the high density group of birds.

TABLE 2. Effect of high density of bird's numbers and the addition of bee pollen to the diet on the differential leucocyte count of quail males.

Treatment	Parameters (Mean \pm SE)				
	Lymphocyte cells %	Heterophil cells %	Basophil cells %	Eosinophil cells %	Monocyte cells %
(G1) Control group(n=21 birds)	49.00 \pm 2.11 ^b	39.50 \pm 1.43 ^{bc}	3.16 \pm 0.40 ^a	2.66 \pm 0.55 ^a	5.66 \pm 0.71 ^a
(G2) Birds numbers 21 and add 20g/kg pollen	54.83 \pm 3.10 ^{ab}	33.33 \pm 2.69 ^{cd}	3.33 \pm 0.33 ^a	2.33 \pm 0.61 ^a	6.16 \pm 0.47 ^a
(G3) Birds numbers 21 and add 30g/kg pollen	47.33 \pm 1.96 ^b	41.00 \pm 2.51 ^b	3.00 \pm 0.36 ^a	2.50 \pm 0.22 ^a	5.83 \pm 0.65 ^a
(G4) High density group (n=75 birds/m ²)	40.00 \pm 3.19 ^c	49.50 \pm 3.01 ^a	2.50 \pm 0.42 ^a	2.66 \pm 0.21 ^a	5.33 \pm 0.84 ^a
(G5) High density group (75 birds/m ²) and add 20g/kg pollen	61.00 \pm 1.57 ^a	27.66 \pm 1.52 ^d	3.33 \pm 0.49 ^a	2.16 \pm 0.47 ^a	5.83 \pm 1.01 ^a
(G6) High density group (75 birds/m ²) and add 30g/kg pollen	52.66 \pm 2.21 ^b	35.33 \pm 2.20 ^{bc}	3.00 \pm 0.57 ^a	2.50 \pm 0.5 ^a	6.00 \pm 0.73 ^a

Values using different letters in the column are significantly different $P<0.05$.

TABLE 3. Effect of the high density of bird numbers and the addition of bee pollen to the diet on the levels of total proteins, albumin and globulin of quail males.

Treatment	Mean (g) ± SE		
	Total Proteins (g/dL)	Albumin (g/dL)	Globulin (g/dL)
(G1) Control group(n=21 birds)	6.33 ± 0.39 ^a	0.93 ± 0.02 ^a	5.40 ± 0.40 ^a
(G2) Birds numbers 21 and add 20g/kg pollen	5.34 ± 0.50 ^a	0.70 ± 0.09 ^{ab}	4.64 ± 0.49 ^a
(G3) Birds numbers 21 and add 30g/kg pollen	6.02 ± 0.30 ^a	0.79 ± 0.03 ^{ab}	5.23 ± 0.30 ^a
(G4) High density group (n=75 birds/m ²)	5.71 ± 0.33 ^a	0.52 ± 0.01 ^c	5.18 ± 0.32 ^a
(G5) High density group (75 birds/m ²) and add 20g/kg pollen	5.73 ± 0.37 ^a	0.66 ± 0.007 ^b	5.07 ± 0.37 ^a
(G6) High density group (75 birds/m ²) and add 30g/kg pollen	6.05 ± 0.35 ^a	0.64 ± 0.04 ^b	5.41 ± 0.35 ^a

Values using different letters in the column are significantly different P<0.05.

TABLE 4. Effect of high density of bird's numbers and the addition of bee pollen to the diet on the levels of heat shock proteins 90, malondialdehyde and glutathione of quail males

Treatment	Mean (g) ± SE		
	Heat shock protein 90 (ng/ml)	Malondialdehyde (μmol/L)	Glutathione (μmol/L)
(G1) Control group(n=21 birds)	3.93 ± 0.29 ^b	0.51 ± 0.01 ^{ab}	5.44 ± 0.49 ^a
(G2) Birds numbers 21 and add 20g/kg pollen	3.34 ± 0.32 ^b	0.50 ± 0.06 ^{ab}	5.91 ± 1.05 ^a
(G3) Birds numbers 21 and add 30g/kg pollen	3.78 ± 0.22 ^b	0.64 ± 0.03 ^b	6.01 ± 0.79 ^a
(G4) High density group (n=75 birds/m ²)	5.37 ± 0.51 ^a	0.79 ± 0.06 ^a	2.71 ± 0.23 ^b
(G5) High density group (75 birds/m ²) and add 20g/kg pollen	3.84 ± 0.30 ^b	0.48 ± 0.04 ^c	5.40 ± 0.7 ^a
(G6) High density group (75 birds/m ²) and add 30g/kg pollen	3.97 ± 0.30 ^b	0.62 ± 0.04 ^{ab}	5.02 ± 0.77 ^a

Values using different letters in the column are significantly different P<0.05.

Table (5) indicates that there was no significant difference ($P > 0.05$) in the level of alanine aminotransferase and aspartate aminotransferase, and the weight of the liver in a group of 21 birds/m² supplemented with 20 and 30 g of bee pollen led to a significant increase in the number of birds ($P \leq 0.05$) in the level of alanine aminotransferase, but it did not lead to a significant difference in the level of aspartate aminotransferase or liver weight compared to the control group. The addition of 20

and 30 g of bee pollen to the diet of the density group of birds did not significant difference ($P > 0.05$) in the above criteria compared to the density group of birds that were not given bee pollen.

Discussion

The high density of birds caused a significant decrease in hemoglobin concentration, packed cell volume, and mean corpuscular hemoglobin concentration, accompanied by a rise in the

TABLE 5. Effect of high density of bird's numbers and the addition of bee pollen to the diet on the levels of alanine aminotransferase and aspartate aminotransferase and liver weight of quail males

Treatment	Mean (g) ± SE		
	Alanine aminotransferase (IU/L)	Aspartate aminotransferase (IU/L)	Liver weight (gm/100g body weight)
(G1) Control group(n=21 birds)	36.74± 11.74 ^b	64.90 ± 6.31 ^a	1.58 ± 0.03 ^{ab}
(G2) Birds numbers 21 and add 20g/kg pollen	54.51 ± 8.98 ^{ab}	70.29 ± 2.23 ^a	1.40 ± 0.03 ^b
(G3) Birds numbers 21 and add 30g/kg pollen	42.62 ± 0.34 ^{ab}	68.44 ± 5.33 ^a	1.46 ± 0.08 ^b
(G4) High density group (n=75 birds/m ²)	74.59 ± 1.65 ^a	73.95 ± 7.57 ^a	1.66 ± 0.07 ^a
(G5) High density group (75 birds/m ²) and add 20g/kg pollen	48.40 ± 9.01 ^{ab}	70.22 ± 6.81 ^a	1.59 ± 0.07 ^{ab}
(G6) High density group (75 birds/m ²) and add 30g/kg pollen	48.01 ± 9.86 ^{ab}	64.23 ± 4.80 ^a	1.41± 0.08 ^{ab}

Values using different letters in the column are significantly different P<0.05.

stress index. The findings are consistent with those of some researchers [19] who discovered a decrease in hemoglobin concentration, packed cell volume percentage, and mean corpuscle hemoglobin concentration when the birds were subjected to transport stress. This may be due to a breakdown in the number of red blood cells or a decrease in their composition, which leads to a decrease in their number and size, which is reflected in the concentration of hemoglobin and packed cell volume [20, 21]. Also, the result was identical to that of the researchers, Selvam et al. [22] who recorded an increase in the percentage of heterophils / lymphocytes, and the reason may lie in the high indicators of stress in the blood of chickens due to the high level of corticosterone hormone [23]. According to Abou El-Soud et al. [24], the H/L rate is measured by the physiological change in organs such as the atrophy of the thymus gland, which produces glucocorticoids, and this gland is affected by corticosterone, as corticosterone causes the release of heterophils, and thus the stress-induced increase in the ratio of H: L is attributed to the effect of stress which increases corticosterone levels.

The results showed that there was no significant difference in the concentration of hemoglobin, MCHC, and PCV, when adding 20 g and 30 g of bee pollen/kg diet to the group of 21 birds. The results agreed with researchers Shaddel-Tili et al. [25] where bee products did not affect red blood

cell count, hemoglobin concentration, and PCV in broiler chickens, and the results did not agree with what was found by Omar et al. [26] who recorded an increase in the number of red blood cells, hemoglobin concentration, and PCV for rabbits that were given 250 g and 500 g of bee pollen. Perhaps the explanation of the reason for the insignificant blood values of our results is the dose used for bee pollen. It was a balanced dose that did not change the blood values and kept them in their normal form, as well as the time period during which the pollen was given was a good period that did not affect the normal blood values in addition to the conditions of the experiment.

The present results showed a significant increase in hemoglobin concentration when adding 20 and 30 gm of bee pollen to a group of birds with a high density of birds. This result was in agreement with the results conducted by researchers Farag and El-Rayes [27] on chicks fed on 0.6% of bee pollen, where the highest values of hemoglobin and red blood cells were recorded. The reason may be that bee pollen contains minerals such as iron, copper, folic acid, and vitamin C and these minerals and vitamins play a major role in the processes of red blood cell formation and maturation [28], as vitamin C activates the secretion of the factor responsible for the formation of red blood cells, erythropoietin from the kidney, and this stimulates bone marrow, influencing the production of red blood cells [29].

The results of MCHC did not agree when adding 30 g of bee pollen to the high density group with researchers Oghenebrorhie et al. [30] where they found that there was no significant difference in MCHC in broiler chickens, and the reason for this was that there was no significant effect on hemoglobin concentration.

The results showed a significant decrease in the number of lymphocytes and a significant increase in the number of heterophil cells when the number of birds increased by 75 birds/m², and the results were identical to a previous study by researchers Singh et al. [31] in which they found decreased lymphocyte counts in chicken blood sample smears which were accompanied by an increased number of heterophil cells in response to stress.

The results of the current study showed a significant decrease in the number of heterophil cells and an increase in the number of lymphocytes when adding 20 g and 30 g of bee pollen to the high density group, and the result was identical to what they came up with. Abdel Salam et al. [32] found when adding bee pollen at a dose of 400, 600, and 800 mg/kg to the diet led to a significant decrease in the number of heterophils and an increase in the number of lymphocytes when compared to the control group, and the reason for these results can be attributed to the presence of amino acids, vitamins, and essential minerals in bee pollen that can enhance the reproduction of immune cells.

The results of the statistical analysis did not show a significant difference in the percentage of the number of lymphocytes and heterophil cells in the group of bird's number 21 birds / m² and added to it 20 and 30 g of bee pollen / kg of diet, the results agreed with what the researchers found Hosseini et al. [33] where the levels of lymphocytes and heterophils in the blood were not statistically different between groups when 9.1% of bee pollen was added to broiler chickens. There was no significant difference in the percentage of numbers of eosinophil, basophils, and monocyte cells between groups. The results agreed with Abuoghaba [34], where the percentages of numbers of eosinophil, basophils and monocyte cells were not affected by roosters fed 500, 1000, 1500 mg of bee pollen, and perhaps the reason is due to the type of birds used in the experiment, as the quail is one of the birds that are resistant to diseases and to difficult and abnormal conditions [35].

The results of our study recorded a significant decrease in the level of albumin in the density group of birds at 75 birds / m² compared with the control group and this result agreed with a previous study conducted by researchers Erisir and Erisir [36] that the density of higher quail birds lowered the albumin level and the cause was attributed to stress.

The addition of bee pollen at a dose of 20 and 30 g/kg diet to the group high density of birds led to a significant increase in the level of albumin compared to the group density of birds that were not given bee pollen. Our results agreed with the findings of the researchers Farag and El-Rayes [27] where an increase in the level of total proteins and albumin was recorded in chicks fed on diets containing 0.6% of bee pollen, and perhaps the reason for this is that bee pollen possesses high concentrations of protein 22.7% and essential amino acids found in bee pollen [37].

There were no significant differences in the levels of total proteins and globulin between groups, which agreed with the findings of Abuoghaba [34], who found no significant differences in serum total protein and globulin in laying hens treated with pollen bees, indicating improved kidney and liver function, which could be reflected in the balanced nutritional and antioxidant profiles of bee pollen [38].

The current findings revealed a significant increase in the concentration of heat shock proteins in the bird high density group (75 birds/m²). This finding is consistent with the findings of a recent study conducted by Hassan and Asim [39] on broiler chickens subjected to heat stress, which led to a significant increase in heat shock proteins Hsp70 compared to the control group, and Perhaps the reason is that when some birds are subjected to heat stress, the synthesis of most proteins is delayed, but a group of conserved proteins (heat shock proteins) are largely synthesized in response to stress, as Hsp in stressed cells binds to heat-sensitive proteins and protects them from degradation [40].

The results showed a significant increase in malondialdehyde levels accompanied by a significant decrease in glutathione levels in the high density group birds, and our findings were confirmed by the researchers Abo Ghanima et al. [7], where they recorded the negative effects of high

bird density that caused stress to broiler chickens, as the level of malondialdehyde increased and the activity of glutathione in the blood serum decreased. The reason may be attributed to the fact that crowding leads to increased fighting between birds, which causes disturbances in the metabolism, resulting in the induction of stress, the generation of malondialdehyde, reduced activity of antioxidant enzymes, and increased oxidative stress [41].

The current results show that the addition of bee pollen at a dose of 20 and 30 g/kg to the group of birds (75 birds/m²) significantly decreased the level of Hsp 90. The results were identical to what was stated by Hosseini et al. [33] where heat stress biomarkers were reduced, including the level of Hsp in broiler chickens for groups given bee pollen. The researchers investigated by Bongiovanni et al. [42] examined the effect of natural antioxidants against stress and summarized their findings that the dietary inclusion of flavonoids present in bee pollen has a protective effect against acute heat stress.

The present results indicate that the addition of bee pollen at a dose of 20 and 30 g/kg to the group of birds of 75 birds/m² had a significant decrease in the level of malondialdehyde, accompanied by a significant increase in the level of glutathione. The results were identical to what the researchers found by Zweil et al. [43] who confirmed that feeding Japanese quail with different levels of bee products (propolis and bee pollen) led to a decrease in the level of malondialdehyde and an increase in the level of glutathione, and this may indicate the activity of strong antioxidants in bee pollen, especially phenols, where bee pollen is considered a powerful natural antioxidant because of its biological components, especially polyphenols and phenolic acid derivatives. These compounds have various biological properties, such as antioxidants, anti-inflammatory, and anti-carcinogenic [44].

The results showed that there was no significant difference in the level of alanine aminotransferase and aspartate aminotransferase in the group of 21 birds/m² supplemented with 20 and 30 gm of bee pollen compared to the control group, and when 20 and 30 gm of bee pollen were added to the bird population density group, Similarly the results of Martiniakova et al. [45] showed no significant difference in ALT levels when bee pollen was

added and, on the contrary, lower values of AST were shown in the 0.5% and 0.75% groups of bee pollen. Perhaps the reason is that bee pollen improves liver function and reduces liver damage, and perhaps the protective effect of bee pollen on the liver is due to the antioxidant content of some flavonoids such as quercetin, which plays a role as an antioxidant against oxidants that cause liver damage [46].

The increase in the density of birds' numbers led to a significant increase in the level of alanine aminotransferase enzyme, and the results were identical to the results that showed that stress increases serum levels of AST and ALT in broiler chickens [47]. Other studies showed no significant difference in serum AST and ALT concentrations between groups [48]. The increase in ALT may be due to some harmful effects of heat stress that may occur on liver activity [49].

The results of the current study did not show a significant difference in the relative weight of the liver for all groups, as the results were in agreement with Tong et al. [50] where they confirmed that the high population density of chickens did not affect the weights of the liver and spleen, and the results were identical with Hosseini et al. [33] who did not observe any effect of bee pollen on the weight of the pancreas, liver, and heart in broiler chickens. While the results of the two researchers, Hassan and Asim, [39] did not match, as the result of their study showed that exposure to heat stress reduces the weight of the liver. Perhaps the conditions of the experiment, the type and sex of the bird, the doses used, and the area in which the birds were placed had a role in the non-occurrence of significant statistical changes.

Conclusion

The density of bird numbers had a negative impact on the health of birds as it caused them stress, and when adding bee pollen improved the immunity of birds, blood picture, and reduced stress.

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Conflicts of interest

The researchers note that there is really no conflict of interest.

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References

1. Greene, S.A. and Thurmon, J.C. Xylazine—a review of its pharmacology and use in veterinary medicine. *Journal of Veterinary Pharmacology and Therapeutics*, **11**(4), 295-313(1988).
2. Estevez, I. Density allowances for broilers: where to set the limits. *Poultry Science*, **86**(6), 1265-1272(2007). Doi:10.1093/ps/86.6.1265.
3. Kamboh, A.A., Hang, S.Q., Bakhhtgul, M. and Zhu, W.Y. Effects of genistein and hesperidin on biomarkers of heat stress in broilers under persistent summer stress. *Poultry Science*, **92**(9), 2411-2418 (2013). Doi: 10.3382/ps.2012-02960.
4. Zhang, Y.R., Zhang, L.S., Wang, Z., Liu, Y., Li, F.H., Yuan, J.M. and Xia, Z.F. Effects of stocking density on growth performance, meat quality and tibia development of Pekin ducks. *Animal Science Journal*, **89**(6), 925-930(2018). Doi:10.1111/asj.12997.
5. Nadia M.A. A study of some physiological, productive and reproductive parameters of Japanese quail under stress condition (Doctoral dissertation, PhD. thesis Department of poultry production, Faculty of Agriculture, El-Fayoum. Cairo University) (2003).
6. Cetin, N. Effect of humate and organic acids supplementation to ration on some hematological parameters in laying hens. *Veterinary Journal of Ankara University (Turkey)*, **53**(3), 165-168(2006).
7. Abo Ghanima, M.M., El-Hack, A., Mohamed, E., Taha, A.E., Tufarelli, V., Laudadio, V. and Naiel, M.A. Assessment of stocking rate and housing system on performance, carcass traits, blood indices, and meat quality of french pekin ducks. *Agriculture*, **10**(7), 273 (2020).
8. Dudley, R.E., Svoboda, D.J. and Klaassen, C.D. Acute exposure to cadmium causes severe liver injury in rats. *Toxicology and Applied Pharmacology*, **65**(2), 302-313(1982). Doi: 10.1016/0041-008X (82)90013-8.
9. Fazayeli-Rad, A.R., Afzali, N., Farhangfar, H. and Asghari, M.R. Effect of bee pollen on growth performance, intestinal morphometry and immune status of broiler chicks. *European Poultry Science*, **7**(1),79-82(2017). DOI:10.15414/jmbfs.2017.7.1.79-82
10. Abdelnour, S.A., Abd El-Hack, M.E., Alagawany, M., Farag, M.R. and Elnesr, S.S. Beneficial impacts of bee pollen in animal production, reproduction and health. *Journal of Animal Physiology and Animal Nutrition*, **103**(2), 477-484(2019). Doi:10.1111/jpn.13049.
11. Wan, S. K., Zhang, C. X., Lin, C. R., Zuo, S. Y. and Qian, J. F. Effect of polysaccharide from buckwheat bee pollen on immune function, blood lipids and anti-oxidation ability on broilers. *Feed Industry*, **38**,11–15 (2013).
12. National Research Council. Nutrient Requirement of Poultry. 9th Ed., National Academy Press, Washington DC. USA. ISBN-13: 978-0-309-04892-7, pp: 176 (1994).
13. Gibson, R.M., Bailey, C.A., Kubena, L.F., Huff, W.E. and Harvey, R.B. Ochratoxin A and Dietary Protein. 1. Effects on Body Weight, Feed Conversion, Relative Organ Weight, and Mortality in Three-Week-Old Broilers. *Poultry Science*, **68**(12),1658-1663 (1989).
14. Drabkin, D.L. and Austin, J.H. Spectrophotometric studies: V. a technique for the analysis of undiluted blood and concentrated hemoglobin solutions. *Journal of Biological Chemistry*, **112**(1), 105-115(1935).
15. Campbell, T.W. *Avian Hematology and Cytology*, 2nd ed., Iowa State University Press. (1995).
16. Gross, W.B. and Siegel, H.S. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Diseases*, **27**(4), 972-979(1983).
17. Burtis, C.A. *Tietz Textbook of Clinical Chemistry*. Saunders (1999).
18. Wierusz-Wysocka, B., Wysocki, H., Byks, H., Zozulińska, D., Wykretowicz, A. and Kaźmierczak, M. Metabolic control quality and free radical activity in diabetic patients. *Diabetes Research and Clinical Practice*, **27**(3),193-197(1995). Doi: 10.1016/0168-8227(95)01043-D. *Egypt. J. Vet. Sci.* **Vol. 54**, No. 1 (2023)

19. Schwartzkopf-Genswein, K.S., Faucitano, L., Dadgar, S., Shand, P., González, L.A. and Crowe, T.G. Road transport of cattle, swine and poultry in North America and its impact on animal welfare, carcass and meat quality: A review. *Meat Science*, **92**(3), 227-243 (2012). Doi: 10.1016/j.meatsci.2012.04.010.
20. Hillman, P.E., Scott, N.V. and Tienhoven, A.V. Physiological responses and adaptations to hot and cold environments. *Stress Physiology in Livestock. Volume III. Poultry*. pp.1-71. (1985).
21. Altan, Ö. Altan, A.L.I., Cabuk, M. and Bayraktar, H. Effects of heat stress on some blood parameters in broilers. *Turkish Journal of Veterinary & Animal Sciences*, **24**(2), 145-148 (2000).
22. Selvam, R., Saravanakumar, M., Suresh, S., Sureshbabu, G., Sasikumar, M. and Prashanth, D. Effect of vitamin E supplementation and high stocking density on the performance and stress parameters of broilers. *Brazilian Journal of Poultry Science*, **19**, 587-594(2017).
23. Qaid, M., Albatshan, H., Shafey, T., Hussein, E. and Abudabos, A.M. Effect of stocking density on the performance and immunity of 1-to 14-d-old broiler chicks. *Brazilian Journal of Poultry Science*, **18**,683-692(2016).
24. Abou El-Soud, S.B., Ebeid, T.A. and Eid, Y.Z. Physiological and antioxidative effects of dietary acetyl salicylic acid in laying Japanese quail (*Coturnix japonica*) under high ambient temperature. *The Journal of Poultry Science*, **43**(3),255-265(2006). Doi: 10.2141/jpsa.43.255.
25. Shaddel-Tili, A., Eshratkhal, B., Kouzehgari, H. and Ghasemi-Sadabadi, M. The effect of different levels of propolis in diets on performance, gastrointestinal morphology and some blood parameters in broiler chickens. *Bulgarian Journal of Veterinary Medicine*, **20**(3),215-224(2017). DOI:10.15547/bjvm.986
26. Omar, M., Hassan, F. and El-Shahat, M. The effects of bee pollen on performance and economic efficiency of New Zealand White rabbits reared under high stocking density. *Damanhour Journal of Veterinary Sciences*, **5**(1), 18-23(2020). Doi: 10.21608/DJVS.2020.156538.
27. Farag, S.A. and El-Rayes, T. Research article effect of bee-pollen supplementation on performance, carcass traits and blood parameters of broiler chickens. *Asian J. Anim. Vet. Adv.*, **11** (3), 168-177(2016). Doi: 10.3923/ajava.2016.168.177.
28. El-Wafa, S.A., Sedki, A.A. and Ismail, A.M. Response of growing rabbits to diets containing black seed, garlic or onion as natural feed additives. *Egypt. J. Rabbit Sci.*, **12**(1), 69-83(2002).
29. Kassab, A., Al-Senied, A.A. and Injida, M.H. Effect of dietary ascorbic acid on the physiology and performance of heat stressed broilers. Proceeding of 2nd Symposium, Ascorbic acid in domestic animals. Ittingen, Switzerland. 270-285 (1992).
30. Oghenebrorhie, O. and Oghenesuvwe, O. Performance and hematological characteristics of broiler finisher fed Moringa oleifera leaf meal diets. *Journal of Northeast Agricultural University (English Edition)*, **23**(1), 28-34 (2016). Doi:10.1016/S1006-8104(16)30029-0.
31. Singh, K.D., Pramanik, P.S. and Kumar, R. Effect of Stocking Density on Stress Reaction and Mortality in Broiler Chickens. *Int. J. Curr. Microbiol. App. Sci.*, **7**, 182-189 (2018).
32. Abdel Salam, A.M., Abd ElAzim, A., Othman, A.M., Makram, A. and Omar, E.M. Effect of dietary propolis supplementation on immunocompetence parameters of Cobb broiler chicks. *Egyptian Journal of Nutrition and Feeds*, **22**(2 Special), 209-213 (2019). Doi: 10.21608/EJNF.2019.103491.
33. Hosseini, S.M., Vakili Azghandi, M., Ahani, S. and Nourmohammadi, R. Effect of bee pollen and propolis (bee glue) on growth performance and biomarkers of heat stress in broiler chickens reared under high ambient temperature. *Journal of Animal and Feed Sciences*, **25**(1),45-51(2016). Doi: 10.22358/jafs/65586/2016.
34. Abuoghaba, A.A.K. Impact of bee pollen supplementation on productive performance, some hematological parameters, blood constituents and semen physical characteristics of Sinai chickens. *Egyptian Poultry Science Journal*, **38**(2), 621-635(2018).

35. Nassar, S.A., Mohamed, A.H., Soufy, H., Nasr, S.M. and Mahran, K.M. Immunostimulant effect of Egyptian propolis in rabbits. *The Scientific World Journal*, **2012**, Article ID 901516 (2012). <https://doi.org/10.1100/2012/901516>
36. Erisir, M. and Erisir, Z. Changes in some biochemical blood parameters of quails (*Coturnix coturnix japonica*) with increasing stocking density. *Turkish Journal of Veterinary & Animal Sciences*, **26**(3),491-496 (2002).
37. Kostić, A.Ž., Milinčić, D.D., Barać, M.B., Ali Shariati, M., Tešić, Ž.L. and Pešić, M.B. The application of pollen as a functional food and feed ingredient—the present and perspectives. *Biomolecules*, **10**(1), 84 (2020).
38. Šarić, A., Balog, T., Sobočanec, S., Kušić, B., Šverko, V., Rusak, G., Likić, S., Bubalo, D., Pinto, B., Reali, D. and Marotti, T. Antioxidant effects of flavonoid from Croatian *Cystus incanus* L. rich bee pollen. *Food and Chemical Toxicology*, **47**(3), 547-554 (2009). Doi: 10.1016/j.fct.2008.12.007
39. Hassan, A.A. and Asim, R.A. Effect of vitamin C and acetylsalicylic acid supplementation on some hematological value, heat shock protein 70 concentration and growth hormone level in broiler exposed to heat stress. *Iraqi Journal of Veterinary Sciences*, **34**(2), 357-363(2020). Doi: 10.33899/ijvs.2019.125950.1195
40. Schlesinger, M. J. Heat shock proteins. *J. Cell Biol.*, **103**,321–325 (1986).
41. Simsek, U.G., Dalkilic, B., Ciftci, M. and Yuce, A. The influences of different stocking densities on some welfare indicators, lipid peroxidation (MDA) and antioxidant enzyme activities (GSH, GSH-Px, CAT) in broiler chickens. *Journal of Animal and Veterinary Advances*, **8**(8), 1568-1572 (2009).
42. Bongiovanni, G.A., Soria, E.A. and Eynard, A.R. Effects of the plant flavonoids silymarin and quercetin on arsenite-induced oxidative stress in CHO-K1 cells. *Food and Chemical Toxicology*, **45**(6), 971-976(2007). Doi: 10.1016/j.fct.2006.12.002.
43. Zweil, H.S., Zahran, S.M., Abd El Rahman, M.H., Desoky, W.M., Abu Hafsa, S.H. and Mokhtar, A.A. Effect of using bee propolis as natural supplement on productive and physiological performance of Japanese quail. *Egyptian Poultry Science Journal*, **36**(1), 161-175(2016). Doi: 10.21608/EPSJ.2016.33248
44. Martín-Muñoz, M.F., Bartolome, B., Caminoa, M., Bobolea, I., Ara, M.G. and Quirce, S. Bee pollen: a dangerous food for allergic children. Identification of responsible allergens. *Allergologia et Immunopathology*, **38**(5),263-265(2010).Doi: 10.1016/j.aller.2009.12.003.
45. Martiniakova, M., Bobonova, I., Toman, R., Galik, B., Bauerova, M. and Omelka, R. Dose-dependent impact of bee pollen supplementation on macroscopic and microscopic structure of femoral bone in rats. *Animals*, **11**(5),1265 (2021). Doi: 10.3390/ani11051265.
46. El-Neney, B.A. and K.H. El-Kholy. Effect of natural additive (bee pollen) on immunity and productive and reproductive performances in rabbits. 1-Growth performance, digestive and immune responses in growing rabbits. *Egyptian Poultry Science Journal*, **34**(2), 579-606 (2014).
47. He, S., Li, S., Arowolo, M.A., Yu, Q., Chen, F., Hu, R. and He, J. Effect of resveratrol on growth performance, rectal temperature and serum parameters of yellow-feather broilers under heat stress. *Animal Science Journal*, **90**(3),401-411(2019). Doi:10.1111/asj.13161.
48. Aslam, M.A., İpek, E., Riaz, R., Özsoy, Ş.Y., Shahzad, W. and Güleş, Ö. Exposure of broiler chickens to chronic heat stress increases the severity of white striping on the pectoralis major muscle. *Tropical Animal Health and Production*, **53**(5),1-10(2021).
49. Banerjee, D., Upadhyay, R.C., Chaudhary, U.B., Kumar, R., Singh, S., Ashutosh, Das, T.K. and De, S. Seasonal variations in physio-biochemical profiles of Indian goats in the paradigm of hot and cold climate. *Biological Rhythm Research*, **46**(2),221-236(2015). Doi:10.1080/09291016.2014.984999.
50. Tong, H.B., Lu, J., Zou, J.M., Wang, Q. and Shi, S.R. Effects of stocking density on growth performance, carcass yield, and immune status of a local chicken breed. *Poultry Science*, **91**(3), 667-673(2012). Doi: 10.3382/ps.2011-01597.

تأثير الكثافة العالية لأعداد الطيور وإضافة حبوب لقاح النحل على مستوى بروتين الصدمة الحرارية ٩٠ ومعايير الدم ذكور السمان

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الهدف من هذه الدراسة هو تحديد دور حبوب لقاح النحل في تحسين معايير الدم وبروتينات الصدمة الحرارية ٩٠ لذكور السمان المعرضة لكثافة عالية لأعداد الطيور. بلغ العدد الكلي (٣٠٠) طائر عمرها ٧ أسابيع موزعة عشوائياً على ٦ مجموعات تجريبية، مدة التجربة ٨ اسابيع. المجموعة الأولى تم وضع ٢١ طائراً / م^٢، والمجموعة الثانية تم وضع ٢١ طائراً / م^٢ وأضيف حبوب لقاح النحل بجرعة ٢٠ غم / كغم علف، والمجموعة الثالثة تم وضع ٢١ طائر / م^٢ وأضيف حبوب لقاح النحل بجرعة ٣٠ غم / كغم علف. والمجموعة الرابعة تم وضع اعداد عالية الكثافة ٧٥ طائر / م^٢، والمجموعة الخامسة تم وضع اعداد عالية الكثافة ٧٥ طائر / م^٢ وأضيف حبوب لقاح النحل بجرعة ٢٠ غم / كغم علف، والمجموعة السادسة تم وضع اعداد عالية الكثافة ٧٥ طائر / م^٢ وأضيف حبوب لقاح النحل بجرعة ٣٠ غم / كغم علف. أظهرت النتائج أن كثافة اعداد الطيور سببت انخفاض معنوي في تركيز الهيموكلوبين ، حجم الخلايا المرصوصة ، معدل تركيز هيموكلوبين الكرية ، عدد الخلايا اللمفية ، مستوى الألبومين ، الكلوتاتيون ، وزيادة معنوية في مؤشر الإجهاد ، عدد الخلايا المتغايرة ، وبروتين الصدمة الحرارية ٩٠ ، و الالنين امينوترانسفيريز. أدت إضافة ٢٠ و ٣٠ غم من حبوب لقاح النحل إلى الطيور عالية الكثافة إلى زيادة معنوية في تركيز الهيموكلوبين ، وعدد الخلايا اللمفية ، والألبومين ، بينما تسبب في انخفاض كبير في مؤشر الإجهاد، وعدد الخلايا المتغايرة، وبروتينات الصدمة الحرارية ٩٠، والمانولدايالديهايد. استنتجت الدراسة أن إضافة حبوب لقاح النحل وحدها لم تؤثر على المعايير الطبيعية لقيم الدم، بينما إضافتها إلى طيور السمان التي تعاني من كثافة اعداد عالية أدت إلى تحسين معايير الدم وتقليل الإجهاد.