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Research article

Effect of dietary supplementation (propolis and/or digestarom) on some blood indexes in broiler chicks under chronic heat stress

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Abstract

This study was conducted to determine the adverse effects of high temperature on some blood parameter of broiler chickens reared under high environmental temperature and evaluating the adding of (propolis and/or digestarom) on diet, three hundred, one-day-old broiler chicks (Rose 308) were distributed equally into two separated room, thermoneutral (TN) groups and heat stressed (HS) group (33 ± 2°C) all over the experiment (42) day. These groups subdivided in to five treated groups (30) chick each. They offered a basal diet supplemented with either propolis (2g/kg of diet), or digestarom (150mg/kg), or a mixture of (propolis 2g/kg +digestarom 150mg/kg) or without any diet supplementation with the vaccine or without any diet supplementation without the vaccine. Alanine Transaminase (ALT), Aspartate Aminotransferase (AST), Total Protein and albumin levels were estimated using commercial kits. The result revealed that dietary supplementation with propolis and /or digestarom caused significant (P≤0.05) increases in blood plasma levels of total protein, globulin, levels of albumin, and significantly reduce (P≤0.05)ALT and AST with or without heat stress as compared to their control groups, while there was a significant reduction in levels of total protein, albumin, globulin (P≤0.05), and significantly increase(P≤0.05 in level of AST and ALT. It has concluded that providing broiler's diets with propolis alone or in combination with digestarom are effective in reducing the adverse effects of heat stress in broilers especially the mixture of propolis with digestarom, which gave the best result.

Keywords: Albumin, AST, ALT, Broiler, Digestarom, Propolis.

Introduction

Heat stress is one of the serious problems affecting poultry breeding and production (1). Broiler chickens are perform well in suitable ambient temperature range 10 and 26c° (2). Broiler chicks appear more sensitive to thermal stress than other bird due to their greater metabolic activity (3). The detrimental effects of heat stress as high mortality, decreased feed intake, lower body weight gain and poor feed efficiency are common in broilers subjected to heat stress (4) moreover, oxidative stress in cells, as a consequence of increased free radical generation reactive oxygen species (ROS) and reflected in increases body temperature (5) moreover HS have immunosuppressant effect and dramatic physiological change in chicken organs (6). Several methods are available to alleviate the effect of high environmental temperature on poultry and as it is impractical and very expensive to cool animal buildings, focused on diet manipulations are one of the solutions which proven their success in ameliorating the negative impact on stressed chickens (7). Propolis is an adhesive, dark yellow to brown colored balsam that smells like resin. It is collected from the buds, leaves and similar

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parts of trees and plants by bees and mixed with their wax (8). Propolis is rich in flavonoid, phenolic acid and terpenoid contents (9) and flavonoids known as antioxidant (10). Propolis supplementation is used in poultry diets because of its anti-oxidative, cytostatic, anti-mutagenic and immunomodulatory properties (8). Phytogenic feed additives are plant extracts originating from the leaves, roots, tubers or fruits of herbs, spices or other plants are used as natural alternatives to antibiotic , having popularity within the feed industry as growth promoters, antioxidant activities ,stimulate immune function and their safety wide rang with fewer hazards. (11).

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 295

Experimental broilers

In this study, three hundred, one -day-old broiler chicks (Ross-308) from a commercial hatchery, Al-Zahra hatchery Al-Qadisiyah province, were used. Chicks they weighed at incubating with (45-50) g. blood samples collected from heart puncture from ten chickens of each group at the end of experiment (42d). Sera used for estimating the total protein, albumin, globulin and liver enzymes (AST, ALT) (indirect method).

The chicks were divided in to two separated room for two trials (150birds) for each TN and HS, each room then being subdivided in to five partitions by plastic obstructions, representing5 treated groups (n=30 chicks)for each group, they were put in floor pens provided with wood-shavings litter and lightening period of 23 h. /day throughout the experimental period.

Nutritional Regimes

a. Composition of experimental diet Starter: 1 to 21 days

Table (1): Feed constituents of starter basal experimental diet.

Constituents	Quantity / ton	Percentage (%)
Corn	465 Kg	46.5 %
Wheat	200 Kg	20%
Soy bean meal	300 Kg	30%
Calcium	17 Kg	1.7 %
Premix 1%	15 Kg	1.5%
Salt	2 Kg	0.2%
Mycofix select	1 Kg	0.1%
Energy %	2940	
Protein %	20.9	

b. Final: It was used from 22 days until the finishing the experiment 45days composed of the following:

Table (2): Feed constituents of final diet.

Constituents	**Quantity / ton**	**Percentage (%)**
Corn	500 Kg	50 %
Wheat	200 Kg	20%
Soy bean meal	265 Kg	26.5%
Calcium	17 Kg	1.7 %
Premix 1%	15 Kg	1.5%
Salt	2 Kg	0.2%
Mycofix select	1 Kg	0.1%
Energy %	2970	
Protein %	19.6	

Experimental design

Thermo neutral (TN) groups reared under the usual heat program which was starting at 33°C then was gradually reduced to 2-3°C per week, reaching 21°C; and maintained until the end of the study.

G1: group offered a corn-soybean meal basal diet with propolis 2g/kg diet along 42 days.

G2: group offered a corn-soybean meal basal diet supplemented with Digestrom®150g/ton along 42 days.

G3: group offered a corn-soybean meal basal 1 diet supplemented with (propolis 2gm/kg diet + 150g/ton (Digestrom®) along 42 days.

G4: Control group offered a corn-soybean only basal diet.

G5: Control (neither vaccinated nor supplemented) group.

Heat stress, (HS) groups were beginning at 33± 2°C and maintained all over the study

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(42 day). The design of the experiments as follow:

HG1: group offered a corn-soybean meal basal diet with propolis 2gm/kg diet throughout the duration of the experiment.

HG2: group offered a corn-soybean meal basal diet supplemented with Digestarom150gm/ton throughout of the duration of the experiment.

HG3: group offered a corn-soybean meal basal diet l supplemented with (propolis 2g/kg diet + 150g/ton (Digestrom®) throughout of the duration of the experiment.

HG4: Control group offered a corn-soybean meal basal diet without supplementation.

HG5: Control (neither vaccinated nor supplemented) group.

Blood Sampling

Blood samples were collected at 42 day from jugular vein using disposable syringe, and then added to test tubes without anticoagulant, left in room temperature for 2 hrs. then centrifuged for 10 min at (3000 rpm). After that, serum separated immediately and kept at 4 ºC until assayed.

Total Protein, Albumin, and Globulin levels:

Plasma total protein was detected according to Bayourat (Biuret technique) using a private kit (RANDOX) (12). The spectrophotometric technique was used to determine, plasma total protein and albumen.

Albumin level:

Albumin concentrations measured using (Bromocresol Green) to estimate the level of albumin in the serum by private kit (TC).

Globulin level:

Globulin level measured indirectly, depending on measuring the concentration of total protein and albumin in the serum.
Globulin magnitude g /dL =Total protein con. –Albumin conc.

Estimation of liver enzyme: (ALT and AST)

Asparate amino transefrase and alanine amino transefrase enzymes activities were measured by using enzymatic kit. This test was done according to instructions of manufactures manual. Component of Aspartate aminotransferase Kit (AST) and (ALT): used to measure the activity of these Yeasts commercial Kit (RANDOX) they were carried out according to the manufactures instruction. The Kit is used only in the event that the spectrophotometer device dose not read wave length (546 nm). Examination was conducted following the instruction recorded of laboratory.

Results

Effects of different treatments and group in total protein (g/dl) at 40 days old broiler chicken.

Results presented in Tables (4) and (5), appear the effect of dietary additive propolis and/or digestarom in the mean value of concentration of serum total protein of broiler at day 40 old subjected to heat stress. There were significant increase ($P<0.05$) in TN treated groups, as well as, in HS treated groups and TG3, TG1 then TG2 in comparing with TG4, TG5 control groups values are (4.837±0.08; 4.069±0.06; 3.838±0.02; 3.718±0.093;. 272±0.14) respectively, higher values found in TG3 . In HS groups significant increase ($P<0.05$) in the mean value of G3, G1, G2 in comparing with G4, G5 with values (3.908±0.03; 3.689±0.042 ; 3.506±0.02; 3.336±0.08; 2.995±0.04) respectively with highest elevation showed in G3 .Comparing among all exposed to heat stress groups, each with the analogous group of TN revealed that there were significant decrease ($P<0.05$) in the mean values between HG1, TG1; HG2 ,TG2; HG3 and TG3 and also between HG4,TG4.

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Table (3): effects of different treatments and group in total protein (g/dl) at 40 days old

Groups	Mean ± SE		LSD value
	Thermo neutral (TN)group	Heat stress(HS)	
G1	4.069±0.06 Ba	3.689±0.04 Bb	0.247
G2	3.838±0.02 Ba	3.506±0.02 BCb	0.127
G3	4.837±0.08 Aa	3.908±0.03 Ab	0.286
G4	3.718±0.09 Ba	3.336±0.08 Ca	0.416
G5	3.272±0.14 Ca	2.995±0.04 Da	0.5
LSD value	0.382	0.212	

Number of samples: 5 from each group. G1: administrated propolis 2 g/kg, G2: digestarom150mg/kg, G3: administrated *mix* (propolis +digestarom), G4: Control Positive, G5: Control Negative. Means having different capital letters (in columns) and small letters (in rows) are significant difference (P<0.05). LSD: less significant differences.

Effects of different treatments and group in Albumin (g/dl) at 40 days old.

Tables (4) and (6) summarized the mean values of concentration of albumin in TN and HS groups. It was recorded of significant increases (P<0.05) in the values of TN as well as HS groups in comparing witty non treated control groups with or without heat stress, TG3,TG1then TG2 in comparing with TG4,TG5 control groups values are (2.774±0.04; 2.174±0.04; 2.064±0.007 (2.028±0.07; 1.728±0.08) respectively and TG3 have the higher value, while in HS groups significant increase (P<0.05) in the mean value of G3,G1,G2in comparing with G4,G5 with values (2.034±0.01; 1.897±0.03; 1.809±0.02; 1.658±0.02; 1.52±0.04) respectively , higher values found inHG3.. Insignificant difference was found between HG1 and HG2.In comparing all of heat stressed groups with all of homologous TN groups revealed the presence of significant decreases (P<0.05) of the values between HG1, TG1;HG2 ,TG2 ;HG3 ,TG3 and between control groups HG4, TG4 and HG5 ,TG5.

Table (4): effects of different treatments and group in Albumin (g/dl) at 40 days old

Groups	Mean ± SE		LSD value
	Thermo neutral TN	Heat stress HS	
G1	2.174±0.04 Ba	1.897±0.03 Bb	0.172
G2	2.064±0.007 Ba	1.809±0.02 Bb	0.084
G3	2.774±0.04 Aa	2.034±0.01 Ab	0.163
G4	2.028±0.07 Ba	1.658±0.02 Cb	0.262
G5	1.728±0.08 Ca	1.52±0.04 Db	0.311
LSD value	0.247	0.117	

G1: administrated propolis 2 g/kg, G2: digestarom150mg/kg, G3: administrated *mix* (propolis +digestarom), G4: Control Positive, G5: Control Negative. Means having different capital letters (in columns) and small letters (in rows) are significant difference (P<0.05). LSD: less significant differences

Effects of different treatments and group in Globulin (g/dl) at 40 days old.

Globulin concentration at day 40 in TN groups showed significant increases (P<0.05) in the values of TN and HS treated groups with or without heat stress. TG3, TG1 and TG2 in comparing with TG4 and TG5. values are (2.062±0.07; 1.895±0.02; 1.774±0.02; 1.69±0.06; 1.544±0.06 respectively. Insignificant differences were

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noticed betweenTG2and TG4. Heat stressed groups G3, G1, G2 and values are (1.874±0.03; 1.792±0.02; 1.696±0.01) in compared to G4 ,G5 (1.677±0.06; 1.475±0.02) respectively.

Table (5): Effects of different treatments and group in Globulin (g/dl) at 40 days old

Groups	Mean ± SE		LSD value
	Normal group	Heat stress	
G1	1.895±0.02 Aba	1.792±0.02 ABa	0.118
G2	1.774±0.02 BCa	1.696±0.01 Ba	0.097
G3	2.062±0.07 Aa	1.874±0.03 Aa	0.258
G4	1.69±0.06 BCa	1.677±0.06 Ba	0.308
G5	1.544±0.06 Ca	1.475±0.02 Ca	0.236
LSD value	0.232	0.158	

Number of samples: 5 from each group. G1: administrated propolis 2 g/kg, G2: digestarom150mg/kg, G3: administrated *mix* (propolis +digestarom), G4: Control Positive, G5: Control Negative. Means having different capital letters (in columns)

Effects of different treatments in level of Aspartate transaminase AST (g/dl) in 40 days old broilers at TN and HS conditions.

Levels of AST were determined in plasma of experimental broilers at 42 day of age. The results summarized in Tables (4) and (8) which indicated that there was significant decreased P<0.05 in the level of AST in sera of treated TN and HS treated groups in comparing with non-treated control groups. TN groups G3, G1, G2 value are (6.74±0.2; 7.32±0.15; 7.5±0.2) respectively, while cont. G4, G5 (8.58±0.15; 7.98±0.06) respectively and the lowest level was found in TG3.Comparing within HS groups appear the presence of a significant significantly decreases (P<0.05) in the level of AST, G3, G1, G2 values are(7.34±0.16; 8.04±0.19; 8.44±0.17) respectively in comparing with G4,G5 (9.36±0.2;9.04±0.16),also significant decrease between HG2 and HG1. Comparing all of heat stressed groups with all homologous TN groups revealed the presence of significant increases (P<0.05) of the levels values of AST between HG1, TG1; HG2, TG2, and HG3, TG3.also between control groups HG4, TG4, HG5 and TG5. The data also showed decrease in the level of AST in all HS groups in comparing to control TN TG4 and TG5.

Table (6): Effects of different treatments and group in AST (g/dl) at 40 days old.

Groups	Mean ± SE		LSD value
	TN	Heat stress HS	
G1	7.32±0.15 Cb	8.04±0.19 Ba	0.569
G2	7.5±0.2 BCb	8.44±0.17 Ba	0.742
G3	6.74±0.2 Da	7.34±0.16 Ca	0.649
G4	8.58±0.15 Ab	9.36±0.2 Aa	0.714
G5	7.98±0.06 Bb	9.04±0.16 Aa	0.399
LSD value	0.554	0.579	

Number of samples: 5 from each group. G1: administrated propolis 2 g/kg, G2: digestarom150mg/kg, G3: administrated *mix* (propolis +digestarom), G4: Control Positive, G5: Control Negative. Means having different capital letters (in columns) and small letters (in rows) are significant difference (P<0.05). LSD: less significant differences.

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Effects of different treatments in level of alanine transaminase ALT (g/dl) in 40 days old broilers at TN and HS conditions:

Levels of ALT were determined in plasma of experimental broilers 42 days of age. The results summarized in (tab. 4.9) which indicated that there was significant decreased (P<0.05) in the level of ALT in sera of treated groups with or without HS. TN groups G3, G1, G2 values are(4.53±0.15; 4.86±0.09 1;4.88±0.15) respectively, and lowest level was found in G3 ,while cont. G4 and G5 (5.96±0.2; 5.4±0.2) respectively .Comparing within HS groups appear significantly decreases (P<0.05) in the level of ALT, G3, G1, G2 in comparing to G4, G5 with values (5.44±0.13; 6.44±0.19;6.86±0. 7.36±0.2 7.1±0.3) respectively with lowest level in G3. Comparing all of heat stressed groups with all homologous TN groups revealed the presence of significant increases (P<0.05) of the levels values of ALT between HG1, TG1; HG2, TG2; HG3 ,TG3, also among control groups HG4, TG4, HG5 and TG5. The data also showed significant increase in the level of ALT inHG1 and HG2 of HS groups in comparing to thermo-neutral control groups TG4 and TG5. While HG3 showed significant decrease in the level of ALT compared to them.

Table (7): Effects of different treatments and group in ALT (g/dl) at 40 days old

Groups	Mean ± SE		LSD value
	Normal group	Heat stress	
G1	4.88±0.15 BCb	6.44±0.19 Ba	0.564
G2	4.86±0.09 BCb	6.86±0.2 Aba	0.644
G3	4.53±0.15 Cb	5.44±0.13 Ca	0.477
G4	5.96±0.2 Ab	7.36±0.2 Aa	0.769
G5	5.4±0.2 Bb	7.1±0.3 ABa	0.862
LSD value	0.522	0.692	

Number of samples: 5 from each group. G1: propolis at a dose 2 g/kg, G2: digestarom150g/ton, G3: administrated *mix* (propolis +digestarom), G4: Control Positive, G5: Control Negative. Means having different capital letters (in columns) and small letters (in rows) are significant difference (P<0.05). LSD: less significant

Discussion

Serum IgG concentration in the chickens fed propolis supplementation reared under both normal and high temperatures were higher than those fed the control diets, indicating that propolis could modulate humeral immunity and supplementation activates the immune system in chickens, increasing macrophage increase activity of B-lymphocytes, which would be able to produce immunoglobulins. Hence, the increased levels of IgG in broilers fed dietary BG are probably related to the B-lymphocyte stimulation by cytokines (13). The increased levels in birds treated with propolis may be related to the stimulation of B lymphocytes by increasing macrophage activity and increasing concentrations of cytokines such as interleukin-1, interleukin-2, and interleukin-4 (14). These cytokines further stimulate B lymphocytes to become plasma cells, producing immunoglobulins (15). These effects could be attributed to the benzene and flavonoids constituents of propolis, found that stressors are able to enhance the immunoglobulin levels even at 24 h after subjection to the stressing conditions. In the present study, the levels of in plasma immunoglobulin levels were decreased in the chickens housed at HSD. These results may be associated with decreased immunological memory, therefore leading to an increase in the susceptibility of

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chickens to pathogenic challenge. These findings showed that serum globulin concentration in the chickens fed dietary propolis supplementation reared under both normal and high temperatures were higher than those fed the control diets, indicating that propolis could modulate humeral immunity and activates the immune system in chickens (16) demonstrated that serum total protein and globulin levels were significantly increased in propolis (2 g/kg). In addition, serum total protein and globulin levels were significantly increased in chickens fed with 2% propolis and this agree (17) and under thermo-neutral temperatures. Finally, anti-oxidizing properties of propolis considered to improve lipid metabolism, liver morphological structures and biological functions (18). This result is in agreement with the studies (19) who concluded that dietary natural antioxidants are the first strategy against the unpleasant consequences of heat stress on the poultry production. Level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined in plasma of experimental broilers 42 day of age, their level in plasma are associated with cellular injury in broilers exposed to high temperature Table (4.10 and Table 4.11 where there are elevation of these two enzymes in HS groups compared to TN groups and this results are in line of many researchers who indicated that these enzymes and other enzymes are used as tools for differential diagnosis in the clinical biochemistry and, the plasma levels of different enzymes are affected as animals or even human subjected to stress (20) the results of present study agreement (21) who indicated that exposure to high ambient temperature markedly increased plasma ALT levels in the broilers after different periods of heat stress, and its level increased significantly as the duration of heat stress was increased. Heat challenge increases body temperature, which consequently leads to an increase in free radical production and subsequent oxidative damage. Increases in plasma LDH, AST, ALT, and CK prolonged heat stress results in a dramatic physiological change in chicken organs and these changes might be used as indicator of heat stress. Since the bulk of each enzyme is located in different tissues, their abnormal levels in the blood plasma can give an indication to specific muscle or organ damages and activity partly attributed to cellular damage (22). Consequence of heat stress under heat stress conditions, the activities of CK, ALT and AST were generally high, on other hands dietary supplementation of propolis 2g/kg of diet or prebiotic 150g/ton or combination of two significantly reduced the two enzymes activity in broiler chickens and this result may reflecting that propolis may regal ate liver metabolism (18). Showed a significant decrease in serum alanine transaminase activity in Japanese quail given 1 g/kg of propolis compared with birds treated with flavomycin. Reduced enzyme activity could indicate that propolis is able to reduce tissue damage and prevent the leakage of enzymes through cellular membranes and due to its antioxidative function (23). Concerning the role of combination of propolis and prebiotic, the result revealed better than each of them alone and the level decreased significantly ($p<0.05$) in birds fed mixed supplementations compared to the control group due to improved liver function and reduced liver damage. This result is in agreement with the studies of (19), whom concluded that dietary natural antioxidants are the first strategy against the unpleasant consequences of heat stress on the quality of poultry meat. The protective effect of propolis upon liver could be due to antioxidant contents of some flavonoids, which play a role as antioxidant against oxidative material, which caused damage to liver (24). In the current study, elevated activity of plasma AST, ALT, after 6- of chronic heat exposure treatment was observed, suggesting possible liver and muscular oxidative injuries in broiler breeders.

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