The immune response of broiler chickens fed diets supplemented with Propolis and Digestarom under heat stress condition

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Summary

This research was carried out to evaluate the effect of dietary supplementation of a mixture of propolis and phytogenic (Digestarom)on immune responses against Newcastle disease and infectious bursal disease , also Heterophil/lymphocyte ratios in vaccinated broiler under thermoneutral (maintained on usual heat program)and heat stress maintained in (33 ± 2°C). total of three hundred, one-day-old broilers (Rose 308) chicks were distributed equally in two separated room, thermoneutral groups and heat stress HS groups along the duration of the experiment (42)day, then each group subdivided in to five groups(30 chicks for each) as follow: (thermoneutral group and heat stress group)fed a basal diet supplemented with propolis (2g/kg of diet),(thermoneutral group 2and heat stress2)fed Digestarom(150mg/kg).(thermoneutral group 3 and heat stress group 3) fed a mixture of (propolis2g/kg +Digestarom150mg/kg) and two control groups without additive. Antibody titers against Newcastle disease and infectious bursal disease were measured and Heterophil/lymphocyte ratio was estimated. In heat stress chickens the results revealed a significant decrease (P<0.05)in immune response with significant increased(P<0.05) in Heterophil/lymphocyte ratio, while higher significant (P<0.05)in antibody titers with significant decrease (P<0.05) in Heterophil/lymphocyte ratio was showed in all thermoneutral chickens but more significant in thermoneutral group 3 and heat stress group 3 groups were gave the best values in comparing with others groups. The role of Propolis or phytogenic on broiler health and immunity had already been reported, but in this study was reported the effects of their mixture on ameliorating the deleterious effects of heat stress.

Keywords: Broiler, Heat stress, immune response, Propolis, phytogenic, Newcastle Disease, Infectious Bursal Disease , Heterophil/lymphocyte ratio.

Introduction

Environmental stressors especially heat stress is one of the problems affecting successful poultry productive and reproductive performance (1). Broiler chickens are homoeothermic, they perform well within a thermoneutral zone range 10 and 26c° of ambient temperature (2). All birds especially broilers seems to be more sensitive to thermal stress due to their greater metabolic activity. Ambient temperatures over thermoneutral (TN) zone of chickens have received more attention, its detrimental effects with heavy economic losses are associated with impaired feed conversion, reduced average daily weight gain, and immunosuppressant increased mortality in growing birds and effect on meat quality and egg productions (3and4). Heat stress (HS) induce immunosuppression of broiler subjected to continuous elevated temperatures (5). Heat stress (HS) depressed circulating antibodies leading to reduced systemic humeral immune response and Cell-mediated immunity

(6), also it affects the developing of immune organs in young chickens, causing decreased relative weights of bursa of Fabricius, thymus and spleen (5). As well as reduced phagocytic ability of macrophages, also decreased numbers of lymphocytes and increase in the numbers of Hetrophils / lymphocyte H: L ratio (7 and 8). As it is expensive to cool poultry buildings, certain nutritional manipulations and several feed additives like propolis, Prebiotics and phytobiotics, Probiotics and some vitamins have been found to be helpful(9 and 10). Propolis (bee glue; BG), it is a natural product, which is collected by honeybees from plants has various biological and therapeutic activities with strong antioxidant properties that improve the growth performance and productivity in birds reared under high ambient temperatures (11 and 12). Phytogenic Digestarom are natural, growth promoters, plant derived substances, natural alternatives to antibiotic, which are incorporated into animal feed to improve performance, productivity and digestibility, elimination of pathogens improving feed conversion and improve immune response (13). This study was set up to determine the effects of heat stress on broiler chicks performance and immune systems, and the effect of adding mixture of dietary propolis and /or phytogenic to alleviate this negative effects .

Materials and methods

In this study, three hundred and one day old, broiler (Ross-308) taken from a commercial hatchery, Al-Zahra hatchery Al-Qadesia province were used. They were weighed 40g, at second day 10 blood samples were collected by heart puncture technique for estimating the maternal immunity against Newcastle disease (ND) and Infectious Bursal Disease (IBD) using Enzyme Linked Immunosorbent Assay (ELISA) (Indirect method).

All chicks of TN(TG1,TG2,TG3,TG4 and TG5) and HS(HG1,HG2,HG3,HG4 and HG5) groups, except TG5 and HG5were vaccinated with live ND vaccine (clone 79-Hepra) via eye drop at one day and (*La Sota* Iso-Vac) via drinking water at day 10, 20, and 30. Also all groups except TG5 and HG5vaccinated with attenuated IBD vaccine (IBDL-Pfizer) at day 13 via drinking water.

Procedure of indirect ELISA test (Synbiotic – USA),this test was done according to the manufacturer's instructions ProFLOK® NDV ELISA Kit (Synbiotic-USA) (14).blood samples were collected from the bird's wing vein of 10 broiler chicken in -each group at 20, and 40 of age. The blood samples (about 3 ml/bird) were collected in tubes and kept at 4°C overnight, serum were separated by centrifugation at 3000 rpm for 5 minutes.

The chicks were distributed in two separated room (150 chick) for each TN and HS, each room then being subdivided in to five partitions by plastic obstructions, representing. Five treated groups (30 chicks for each group) were put in floor pens provided with wood-shavings litter and lightening period of 23 h. /day throughout the experimental period. All experimented chickens fed a corn-soybean meal basal diet and water ad libitum. The temperature in TN was set at 33°C at one day and gradually decreased 2-3°C per week until 21°C, Chicks in HS were kept at constant temperature 33 ± 2°Cuntil 42 days was designated as heat stressed group. Each group treated as follow: (TG1 and HG1) fed a basal diet supplemented with propolis (2g/kg of diet),(TG2 and HG2)fed Digestarom (150mg/kg).(TG3 and HG3) fed a mixture of (propolis 2g/kg +Digestarom®150mg/kg). (TG4 and HG4) Control positive group fed a corn-soybean meal basal diet without supplementation.(TG5 and HG5) Control negative (neither vaccinated nor supplemented) group.

Collected sera were stored at −20 C until further analysis for estimating ND and IBD immune response. Furthermore blood smears was done day 40 for calculating H/L ratios at 42 days. Obtained cell counts were used for calculation of the relative proportion of Hetrophils to lymphocytes (H/L ratio) (15).

Statistical analysis system was adopted to assess the effect of different factors in the studied parameters, using the least significant difference LSD (P<0.05). Multilevel testing to compare the averages of this study, version ratio was calculated (16).

Result and Discussion

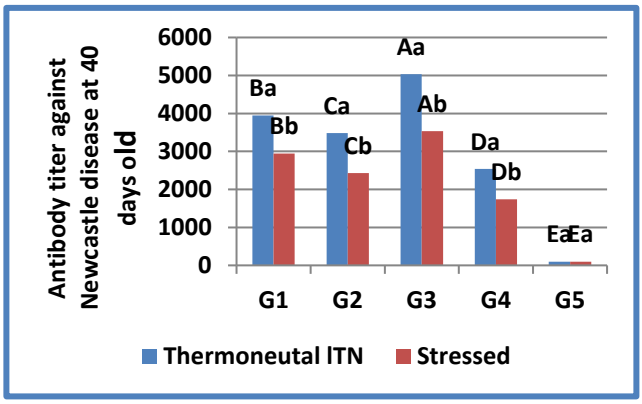
The mean values of maternal immunity revealed good antibodies titer against ND and IBD about 6939.201±121; 9065±141.8 respectively. Maternal antibodies are transferred from hens to the chicks via the egg yolk (passive immunity) (17). The level of maternal antibody decay and its half life time is important information for designing a suitable vaccination program; the estimated half life time of ND maternal antibody level is 6.3 days (18).Table, (1) and (Fig.1) summarized the averages of antibody titers of ND and the effect of different supplements on immune response of broiler chickens 40 day of age, the results of antibodes (Abs) titer showed significant differences (P<0.05) within TN groups and within HS groups in G3 (mixed of prop+ Dig.) was showed the highest level of antibody titers, followed by G1, G2 with values 5033.1±101.9; 3943.4±161.7; 3485.9±162.7 compared to G4 and G5 with values 2537.2±130.1; 0±0 respectively. The role of these additives especially the mixture of (propolis+ Dig.) are appear enhancing the immune response in normal ambient temperature and consequence a good protection will be obtained. propolis or phytogenic each alone may stimulate immune response especially propolis ,which appear more efficient than Digestarom . These results agree with,(19) whom reported that Propolis act as immunomodulatory whether in normal or high ambient temperatures, and this in line well documented the Propolis was rich in bioflavonoid, phenolic acids, vitamins, and phytosterols, promotes faster cell proliferation and differentiation in the immune system, induces T-lymphocyte formation and the division, proliferation and activity of thymus cells, and this study is not agree with.,(20) whom reported that flavonoids have an immunossupressor effect on the lympho proliferative response. The heat stress have negative impact on all heat stressed groupsTable,(1)and(Fag.1).

G4 and G5 showed the lowest significant decrease (P<0.05) of the level of Abs, while the other groups (G3, G1, G2) showed significant increase (P<0.05) of the titer specially G3 which showed the highest level of Abs compared to control groups within HS groups with values 3533.1±237.3; 2943.4±153.3; 2425.9±103.8 respectively, while G4 and G5 1737.2±96.3; 0±0 respectively. The mean value of HS and analogues TN groups revealed were significant decrease (P<0.05) in Abs titer between all groups of HS in comparing to all analogues TN groups. The level of Abs titer of HG3 3533.1±237.3 and HG1 2943.4±153.3 are higher than the level of control group of TN G4 2537.2±130.1which appear the role of the mixed supplements or propolise alone in improving the immune response against ND in spite of heat stress. The mixture of some beneficial feed additive has been already reported as growth promoter and immunomodulatory(10).

Table, 1: Effects of different treatments and group in antibody titer against Newcastle disease at 40 days old

Groups	Mean ± SE		LSD value
	Normal group	Heat stress	
G1	3943.4±161.7 B a	2943.4±153.3 B b	467.82
G2	3485.9±162.7 C a	2425.9±103.8 C b	405.26
G3	5033.1±101.9 A a	3533.1±237.3 A b	542.4
G4	2537.2±130.1 D a	1737.2±96.3 D b	339.9
G5	214.3±11.3 E a	102.7±35.3 D b	NS
LSD value	359.93	402.38	

Number of samples: 10 from each group. Means having different capital letters (in columns) and small letters (in rows) are significant difference (P<0.05). LSD: less significant differences.



Figure,(1): Means of antibody titer against NDV within each of control and stressed for all treatments at 40 d with different capital letters significantly different (P<0.05). Means for each treatment with different small letters significantly different (P<0.05).

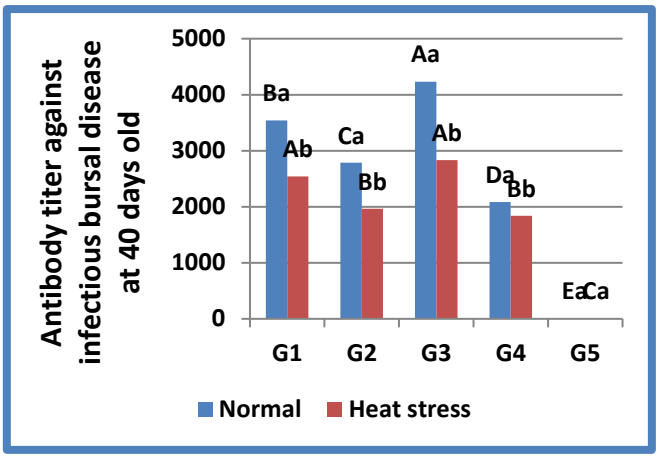
G1:basal diet contain on propolis,**G2**:basal diet contain Digestarom,**G3**:basal diet contain propolis and Digestarom mixture,**G4**:basal diet not contain any feed additive(ve+), Table,(2)and (Fig.2) summarized the effect of different supplements on immune response

Table, 2: Effects of different treatments and group in antibody titer against infectious bursal disease at 40 days old

Groups	Mean ± SE		LSD value
	Normal group	Heat stress	
G1	3543.4±162.5 B a	2543.4±73.3 A b	374.44
G2	2785.9±131 C a	1965.9±208.2 B b	516.6
G3	4233.1±185.8 A a	2833.1±177.9 A b	540.27
G4	2087.2±149.3 D a	1837.2±118.4 B a	400.5
G5	141.6±31.8 D a	105±25.9 D a	
LSD value	403.42	392.22	

Number of samples: 10 from each group. Means having different capital letters (in columns) and small letters (in rows) are significant difference (P<0.05). LSD: less significant differences.

all group vaccination , **G5**:basal diet do not contain any feed additive and not contain any vaccine (ve-).



Figure,(2): Means of antibody titer against IBDV within each of control and stressed for all treatments at 40 d with different capital letters significantly different (P<0.05). Means for each treatment with different small letters significantly different (P<0.05)

against IBD in broiler chickens at 40 day. The results of Abs titer showed significant differences (P<0.05) within all TN groups and within HS groups when compared with control groups of HS or control of TN. The higher level of Abs showed in G3 followed by G1 and G2 then G4 with values 4233.1±185.8; 3543.4±162.5; 2785.9±131; 2087.2±149.3 respectively. The comparison within groups of HS revealed significant increase (P<0.05) in the Abs titer between HG3 and HG2 with value of 2833.1±177.9; 1965.9±208.2 respectively.The higher level Abs was recorded in HG3, HG1 and GH2 in comparing to G4,G5 1837.2±118.4; 105±25.9. The comparison between HS groups and the analogous TN groups shown significant decrease (P<0.05) in the Abs titer in all groups G4 then G2, G1 and G3 with value of 1837.2±118.4; 1965.9±208.2; 2543.4±73.3; 2833.1±177.9 respectively. HG3 and HG1 2833.1±177.9; 2543.4±73.3 revealed a good

protection in heat stressed chickens as they comparing to TG4 2087.2±149.3.

The results indicates the positive effects of Propolis and/or phytogenic in elevating the Abs titer against IBD, also the mixture of prop+ Digestarom® showed the best result compared to each additive alone, and the efficiency of propolis was better than phytogenic. The influence of the heat stress on the broiler bursa of Fabricius and spleen, and other lymphoid organs indicated by atrophy and reduction in bursa weight, these results agree with (5),whom reported the heat stress of influenc on spleen and thymus caused atrophy and reduction in bursa weight known as the main immune organ leading to depressed of defense mechanism against microorganisms and immunosuppression. This could have been a result of the reduction in feed intake, thereby providing less nutrients for the proper development of these organs. Also ,(21) reported that addition of propolis to the diets increased relative weight of bursa of Fabricius and spleen of broiler chickens and improve the immune response, also relate to the ability of Propolis to stimulate and improve immunological function.

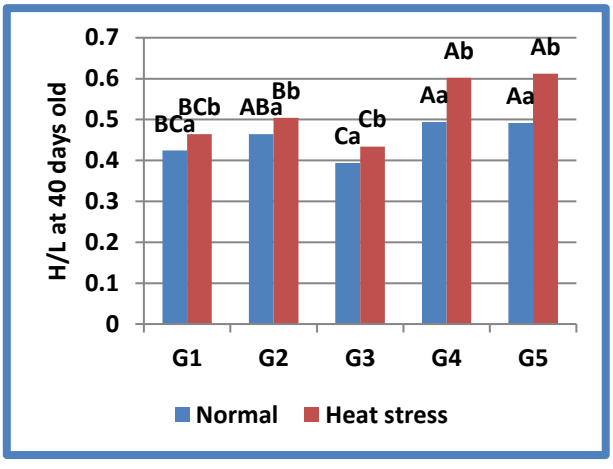
Heterophil/Lymphocyte ratio (H/L) ratio at 40 days old broiler under heat stress.

Table (3) and Fig.3, summarized the level of H/L ratio in broiler at the end of experiment in different treated groups. Reduction in the numbers of lymphocytes and monocytes and increase in the numbers of Hetrophils have been reported for stressed broilers are agreed with,(7) whom reported the dietary application of natural antioxidants is considered an appropriate practical strategy to reduce the deleterious consequences of stressors in animals (11). In this study, an increased Heterophil/lymphocyte ratio was found in heat-stressed chicks without supplementation.

Table, 3: Effects of different treatments and group in H/L at 40 days old

Groups	Mean ± SE		LSD value
	Normal group	Heat stress	
G1	0.424±0.08 BCa	0.464±0.07 BCb	0.037
G2	0.464±0.01 ABa	0.504±0.01 Bb	0.058
G3	0.394±0.01 Ca	0.434±0.03 Cb	0.032
G4	0.494±0.01 Aa	0.602±0.01 Ab	0.061
G5	0.492±0.02 Aa	0.612±0.02 Ab	0.097
LSD value	0.064	0.053	

Number of samples: 5 from each group. Means having different capital letters (in columns) and small letters (in rows) are significant difference (P<0.05). LSD: less significant differences.



Figure, (3) Means of H/L ratio within each of control and stressed for all treatments with different capital letters are significantly different (P<0.05). Means for each treatment with different small letters are significantly different (P<0.05).

TN groups revealed significant decrease (P<0.05) in the H/L ratio in G3, G1, G2.with values (0.394±0.01; 0.424±0.08; 0.464±0.01) respectively, compared to G4,G5 (0.494±0.01;0.492±0.023),this may be due to immunostimulatory effect of propolis or phytogenic or the combination of them which lead to increase proliferation of lymphocyte test these results agree with(7) whom reported and this in line(22)with on the other hand. The HS groups, revealed significant increase (P<0.05) in heat control groups G4,G5(0.602±0.01; 0.612±0.02)respectively in comparing with other heat treated groups G3,G1,G2.(0.434±0.03; 0.464±0.070;504±0.01). The heat stress resulted in elevation of H/L ratio, but the mixed supplement or propolise or phytogenic alone ameliorating the negative impact of heat stress and decrease the elevation in H/L ratio induced by HS. This agree with (23)decrease in Heterophil count is also a positive indication of improved action of dietary antioxidants against heat stress, as indicated in our study stimulation of immune system and reduction of inflammatory reaction of chickens in significant increases in the serum lymphocytes and significant decreases in the numbers of Hetrophils compared with those of the controls groups or HS groups.

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الاستجابة المناعية للدجاج اللاحم المغذى على عليقه مضاف اليها العكبروالدايجستروم تحت ظروف الاجهاد الحراري

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الخلاصة

أجرى هذه البحث لتقييم تأثيرالمكملات الغذائية لمزيج من العكبر والدايجستروم على الاستجابة المناعية ضد مرض نيوكاسل ومرض الجراب المعدية،وأيضا نسب الهيتروفيل/ اللمفاويات في الدجاج اللاحم المحصن تحت الحرارة المعتدلة (الحفاظ على برنامج الحرارة المعتاد) والإجهاد الحراري(الحفاظ على 33 ± 2 درجة مئوية). تم توزيع ثلاثمائة فرخ من افراخ اللاحم (روز 308) بعمر يوم واحد بالتساوي في غرفتين منفصلتين،مجموعة الحرارة المعتدلة ومجموعة الأجهاد الحراري على مدار مدة التجربة البالغة (42) يومًا،ثم قسمت كل مجموعة إلى خمس مجاميع متساوية 30 فرخ لكل مجموعة تم علاج كل مجموعةعلى النحوالتالي: (مجموعة الحرارة الأولى ومجموعة الأجهاد الحراري الاولى) غذيت على نظام غذائي قاعدي يكملها العكبر (2 جم / كجم). (مجموعة الحرارة المعتدلة الثانية ومجموعة الأجهاد الحراري الثانية) غذيت على عليقة تحتوي(الدايجستروم®150مجم / كجم). تغذى (مجموعة الحرارة المعتدلة الثالثة ومجموعة الاجهاد الحراري الثالثة) على خليط من (العكبر2 غم/كغم مع الدايجستروم®150مجم / كجم) ومجموعتان سيطرة بدون إضافات. تم قياس معيارالأجسام المضادة ضد مرض النيوكاسل ومرض الجرب المعدية وتم تقدير نسبة المتغيرات واللمفاوية،كشفت النتائج عن انخفاض معنوي ($P<0.05$) في الاستجابة المناعية مع زيادة معنوية ($P<0.05$) في نسبة المتغيرات و اللمفاوية ،بينما ارتفاع معنوي في معيارالأجسام مع انخفاض معنوي في نسبة المتغيرات اللمفاوية في جميع دجاج الحرارة المعتدلة لكن الأكثرأهمية في مجموعة الحرارة المعتدلة الثالثة وأعطيت مجموعات الاجهاد الحراري الثالثة أفضل القيم في المقارنة مع مجموعات أخرى. وقد تم بالفعل الإبلاغ عن دورالعكبرأوالدايجستروم على المناعة وصحة الدجاج اللاحم ،في هذه الدراسة الحالية اوضحت الدور الرئيسي في تخفيف الآثارالضارة للإجهاد الحراري.

الكلمات المفتاحية: دجاج اللحم،الاجهاد الحراري، الاستجابة المناعية،العكبر،مرض نيوكاسل،التهاب جراب فابريشيا.