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Effects of Different Levels of Artichoke (*Cynara scolymus*) on Growth Performance and Immune Responses of Broilers under Heat Stress

Research Article

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ABSTRACT

This experiment was undertaken to evaluate the effect of dietary level of artichoke (*Cynara scolymus*) on growth performance and immune response of broilers under heat stress (HS). Two hundred forty day-old broiler chicks (Ross, 308) were randomly assigned to one of four dietary treatments with four replicate pens per treatment (15 birds per pen) in a completely randomized design with a 2 × 4 factorial arrangement (4 treatment diets and 2 different temperatures rearing systems). Treatment diets were: 1) control diet, 2) and 3) control diet supplemented with 15 and 30 g artichoke, respectively and 4) control diet supplemented with 300 mg/kg vitamin E. Body weight (BW) and feed conversion ratio (FCR) were not influenced by dietary artichoke ($P>0.05$). In the case of sheep red blood cells (SRBC), there were no significant differences between treatments. However, the control group in HS condition showed lower titer for sheep red blood cells (SRBC) while group receiving 3 percent artichoke showed higher titer ($P<0.05$). There was no significant difference between treatments for Newcastle antibody titer. Regarding lymphoid organs, the weights of bourse and spleen were similar, while liver weight differed between treatments. In this regard, chicks under HS in the control group had lower weight than the other groups. Lymphoid organ weights and antibody responses were significantly reduced under HS. These results indicated that HS severely reduced growth performance and immune response of broilers, whereas the immune response of broilers could be improved by dietary artichoke supplementation under HS.

KEY WORDS artichoke, broiler, growth performance, heat stress, immune response.

INTRODUCTION

Heat stress (HS) is of great concern in the poultry industry. Feed efficiency, growth rate, mortality and other important traits governing productivity in the poultry industry are adversely affected by severe HS. Broilers exposed to 32 ˚C showed a 24% decrease in feed intake by 6 wk of age (Geraert *et al.* 1996). It has been established that high environmental temperatures affect the development of a specific immune response in chickens (Thaxton *et al.* 1968). When chicks were exposed to temperatures ranging from 32.2 to 43 ˚C for a short intermittent period of constant high temperatures or cycling high temperature conditions, the resulting antibody response in sheep red blood cells (SRBC) was significantly reduced. Although limited information is available on the effect of HS on the cell-mediated immune responses, Miller and Qureshi (1991) reported a depression in the phagocytic potential of chicken macrophages under HS. Lymphoid organ weights, primary and secondary antibody responses were also reduced. Artichoke (*Cynara scolymus*) is a herbaceous perennial native from southern Europe, northern Africa and the Canary islands. Artichoke is believed to have several beneficial effects, for example some preparations made from artichokes, encouraged the

functioning of liver and kidneys, especially when there is metabolic stress (Pecht, 1996). Schutz *et al.* (2004) reported that the total phenolic contents of approximately 12 g/kg on a dry matter basis in artichoke pomace is a promising source of phenolic compounds that might be recovered and used as natural antioxidants or functional food ingredients. Artichoke leaves extract has traditionally been used, for jaundice and liver insufficiency and it has been suggested as a harmless yet effective treatment option for hypercholesterolaemia (Pittler *et al.* 2005).

Lack of sufficient evidence showing how broilers would respond immunologically under HS if their diets were supplemented with varying levels of artichoke has necessitated further study. The objective of this experiment was to evaluate the effects of dietary levels of artichoke on growth performance and immunosuppression of broilers raised under HS.

MATERIALS AND METHODS

Experimental design and birds

Two hundred forty day-old broiler chicks (Ross, 308) were randomly assigned to one of four dietary treatments with four replicate pens per treatment (15 birds per pen) in a completely randomized design with a 2 × 4 factorial arrangement (4 treatment diets and 2 different temperatures rearing systems).

Treatment diets were: 1) control diet; 2 and 3) basal diets were supplemented with 2 levels of artichoke (15 and 30 g/kg diet) and 4) basal diet was supplemented with 30 mg/100 g diet vitamin E (Table 1).

Table 1 Ingredients and chemical analysis composition of the starter and grower diets

	Starter[1]	Grower
Corn	57.25	63.30
Sybean meal	37.37	31.49
Oil	1.65	1.97
Dicalcium phosphate	1.41	1.04
Carbonate calcium	1.26	1.33
Vitamin premix[3]	0.25	0.25
Mineral premix[2]	0.25	0.25
NaCl	0.42	0.32
DL-methionine	0.14	0.05
Metabolizable energy (kcal/kg)	2900	3000
Crude protein (%)	20.84	18.75
Ca (%)	0.91	0.84
P (%)	0.41	0.33
Na (%)	0.18	0.14
Lysine (%)	1.15	1.00
Metionin (%)	0.47	0.36
Met + Cys (%)	0.82	0.68

[1] Starter diet used to feed the birds from 0 to 21 days.
[2] Provide per kilogram of diet: vitamin A: 9000 IU; vitamin D_3: 2000 IU; vitamin E: 18 IU; vitamin B_1: 0.82 mg; vitamin B_2: 1.0 mg; vitamin B_3: 3.0 mg; vitamin B_5: 35 mg; vitamin B_6: 3.0 mg; vitamin B_9: 1 mg; vitamin B_{12}: 1.5 mg; vitamin K_3: Coline chloride: 500 mg.
[3] Fe: 25 mg; Mn: 32 mg; Cu: 4 mg; Zn: 11 mg; I: 0.16 mg and Se: 0.2 mg.

The broilers under study were divided into two main groups. Those in the first group were maintained at 26 ± 2 ˚C and the second group at (34±2 ˚C and 75% RH, 6 h/day, 10:00-16:00 h in HS). Throughout the experimental period, the broilers were treated *ad libitum* with continuous light and water supplies. Water and feed were provided *ad libitum*, and feed intake and body weight (BW) were recorded weekly. The experiment was performed for 42 days.

Immunocompetence evaluations

Body weight

The body weight (BW) was recorded for each pen. At the end of experiment, chicks were individually weighed and 2 birds from each pen.

Organ collection

Eight birds of each dietary treatment divided between the two temperatures were euthanized at 42 days of age. Thymus, spleen and bursa of Fabricius were collected and weighted.

Sheep red blood cells (SRBC)

To determine the antibody response to sheep red blood cells (SRBC), a direct hemagglutination assay was used. Antibody responses to bovine serum albumin (BSA) and tetanus toxid (TT) were measured by enzyme-linked immunosorbent assay (ELISA). A direct hemagglutination assay was performed to measure the total antibody (IgM and IgG) response to SRBC in serum. Briefly, serum samples were incubated at 56 ˚C for 30 min to inactivate the complement. Fifty microliters of phosphate-buffered saline (PBS) containing 0.05% BSA was dispensed into each well of a round-bottomed 96-well microplate. Serum samples (50 mL) were then added and serially double diluted in the wells from columns 2 to 12. The first column (PBS only) of wells was considered the blank. Then, 50 mL of 1% SRBC in PBS was added to all wells to make a 100 mL final volume. Subsequently, the plates were shaken for 1 min and incubated for 24 h at 37 ˚C to determine agglutination titers. A positive result was recorded when at least 50% SRBC agglutination was observed. To measure anti-SRBC IgG and IgM antibodies, serum samples were treated with 0.2 M 2-mercaptoethanol (2-ME) for 30 min at 37 ˚C. This treatment inactivates IgM, and as a result, hemagglutination observed after treatment with 2-ME is mostly due to the presence of IgG antibodies. The difference between total antibody and IgG titers determines the IgM titer. The procedure was based on Haghighi (2005) results.

ND-HI titer

Newcastle disease (ND) hemagglutination inhibition (HI) titer was determined by the following procedure: a 2-fold

serial dilution of serum was made in a 96-well microlitre plate with V-shaped bottom, containing 25 µL of buffer with 7.2-7.4 pH and 25 µL of serum in all wells. Then, 25 µL of ND virus antigen were added to all wells except those in the last row (controls). Serum dilutions ranged from 1:2 to 1:2.048. The antigen serum mixture was incubated for 10 min at 37 ˚C. 50 µL of 0.5% erythrocyte suspension were then added to each well and the wells were reincubated for 30 min. A positive serum, a negative serum, erythrocytes and antigens were also included as controls. The highest dilution of serum causing complete inhibition of erythrocyte agglutination was considered at the end point. The geometric mean titer was expressed as reciprocal log2 values of the highest dilution that displayed anti-ND-HI (Dong *et al*. 2007).

Statistical analysis

The trial was conducted as a complete randomized design with a 2 × 4 factorial arrangement of treatments with four replicates per treatment. The results of this experiment were analyzed using the GLM procedure of SAS (2003) software. Statistical significant differences between treatment means were determined with tukey test. All statements of significance are based on probability of P<0.05.

RESULTS AND DISCUSSION

Growth performance

The effect of different levels of artichoke on growth performance of 42-d-old broilers under HS is shown in Table 2.

Table 2 Body weight (BW, g) and feed conversion ratio (FCR) on birds at 42 day of age

		BW	FCR
Heat stress (34 ˚C)	Control	1941	1.83
	1.5% artichoke	1816	1.88
	3.0% artichoke	1770	2.02
	Vitamin E	1890	1.93
Normal (26 ˚C)	Control	2130	1.78
	1.5% artichoke	1957	1.83
	3% artichoke	1870	1.88
	Vitamin E	2014	1.78
	SEM	0.004	0.4
	P-value	0.8	0.7

SEM: standard error of the means.

Dietary artichoke levels did not significantly influence on BW and feed conversion ratio (FCR) (P>0.05). There was a significant reduction in BW and FCR when the birds were exposed to HS (P<0.05). There was not a significant interaction in broiler's growth performance between dietary artichoke and environmental temperature (P>0.05). The results indicated that supplementation of artichoke and vitamin E is not significant to improve growth performance of 42-d-old broilers under HS.

Lymphoid organ weights

Lymphoid organ weights were expressed as a percentage of BW (Table 3). None of these organs (bursa, spleen or liver) were significantly affected by the level of artichoke in the diet (P>0.05). However, all organ weights were significantly reduced when birds were exposed to HS (P<0.05).

Table 3 Lymphoid organs weight (100 g of total body weight) on birds at 42 day of age

		Bursa	Spleen	Liver
Heat stress (34 ˚C)	Control	0.21	0.13	1.96^{b}
	1.5% artichoke	0.22	0.14	2.14^{ab}
	3.0% artichoke	0.22	0.15	2.27^{ab}
	Vitamin E	0.23	0.14	2.27^{ab}
Normal (26 ˚C)	Control	0.21	0.14	2.59^{a}
	1.5% artichoke	0.21	0.14	2.17^{ab}
	3.0% artichoke	0.23	0.15	2.32^{ab}
	Vitamin E	0.21	0.15	2.36^{ab}
	SEM	0.0005	0.006	0.12
	P-value	0.20	0.70	0.50

SEM: standard error of the means.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Antibody responses

In the case of SRBC, there were no significant differences between treatments as it is shown in Table 4.

Table 4 Sheep red blood cells (SRBC) and Newcastle disease virus-hemagglutination inhibition titer (NDV-HI) measured by $\log_2$ at 42 day

		SRBC	NDV-HI
Heat stress (34 ˚C)	Control	3.62^{b}	2.12
	1.5% artichoke	4.62^{ab}	2.25
	3.0% artichoke	4.87^{ab}	2.50
	Vitamin E	4.75^{ab}	2.62
Normal (26 ˚C)	Control	4.62^{ab}	2.25
	1.5% artichoke	4.62^{ab}	2.50
	3.0% artichoke	5.00^{a}	2.37
	Vitamin E	4.87^{a}	2.62
	SEM	0.50	0.90
	P-value	0.40	0.45

SEM: standard error of the means.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

However, the control group in heat stress condition showed lower titer for SRBC while the group receiving 3.0%. artichoke showed greater amount of titer (P<0.05). There were no significant differences between treatments (P>0.05) on NDV responses.

The data represent the means of log2 of the reciprocal for the last dilution exhibiting agglutination. Titer of total antibody response significantly increased with increasing dietary artichoke and vitamin E (P<0.05).

Birds receiving the diet with 300 mg/kg of vitamin E and 3.0% artichoke had significantly higher titers of SRBC and newcastle disease virus (NDV) than that of those receiving the other diets (p<0.05).

Birds reared under HS conditions showed a significant reduction in both parameters (P<0.05). The results indi-

cated that BW and FCR were not significantly influenced by dietary vitamin E and artichoke.

These results were in agreement with previous reports (Seier and Bragg, 1993), whereas Colnago *et al*. (1984) reported that dietary vitamin E could improve broiler growth performance. The growth performance of 42-days-old broilers was no significantly reduced when the birds were exposed to HS conditions. This result was in accordance with the general trend observed in the HS broilers (Austic, 1985; Geraert *et al*. 1996). It is believed that for every 10 ˚C increase in ambient temperature above 20 ˚C, there is a 17% reduction in feed intake (Austic, 1985). Geraert *et al*. (1996) observed a 14% reduction in BW from 2 to 4 weeks of age and a 24% reduction from 4 to 6 weeks of age when birds were exposed to 32 ˚C, and suggested that the reduced efficiency could be caused by changes in metabolic utilization of nutrients. In this experiment there was no decrease because the high antioxidant such as cynarin and folinic acid in artichoke can be control heat stress. Organ weights were measured as a percentage of BW. The weights of bourse and spleen were similar, while liver weight differed between treatments. In this regard, chicks under heat stress in control group had lower weight than others ($P<0.05$).

However, all organ weights were significantly reduced by HS. These results are consistent with a previous report (Singh *et al*. 2006). These results agreed with the results of Bartlett and Smith (2003) and suggest that the decrease in lymphoid organ weights could have been a result on the reduction in feed intake, thereby providing fewer nutrients for proper development of these organs under HS conditions. Dietary vitamin E and artichoke significantly increased antibody response to SRBC. This finding was similar to the results reported by Singh *et al*. (2006). Both primary and secondary antibody responses were significantly decreased when birds were reared under HS conditions. This response was similar to previous observations (Thaxton and Siegel, 1970; Donker *et al*. 1990; Bartlett and Smith, 2003). Results showed that there was no significant difference between treatments for Newcastle antibody titer. Ebrahimzadeh *et al*. (2012) showed that antibody titers against newcastle disease virus (NDV) and infectious bronchits virus (IBV) at 21 and 42 days of age in broilers fed supplemental Cr were higher than in broiler chickens fed control diets. Zulkifli *et al*. (2000) reported antibody production in young broiler chickens was decreased in HS condition. This reduction could be indirectly due to an increase in inflammatory cytokines under stress, which stimulates the hypothalamic production of corticotrophin releasing factor. However, this is not always the case. Along with the immune suppression observed in these studies, there has been evidence of immunostimulation (Kelley, 1985; Siegel, 1987), as well as no response (Regnier *et al*. 1980). Due to the considerable variation found in the above-mentioned responses, these researchers believe that there are apparent differences in susceptibility to HS based on genetic lines. These differences may have implications during the application of selection programs for immune responsiveness. Another explanation could be the decrease in feed intake and feed efficiency by HS during this study, leading to a reduction in the nutrients available to mount an effective immune response. In this study, supplementing artichoke in the diet could improve antibody responses when birds were under HS conditions.

CONCLUSION

In conclusion, the results of the present study suggest that supplementation of vitamin E and artichoke in the diet can improve immunocompetence of broilers when reared under HS conditions.

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