



Effects of supplemental ascorbic acid on humeral immune response in broilers reared under heat-stress condition

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Abstract

An experiment was conducted to evaluate the efficacy of supplemental ascorbic acid (Vitamin C) on the haemagglutination inhibition (HI) and enzyme linked immune sorbent assay (ELISA) antibody titer of the commercial broiler chicks vaccinated against Newcastle disease (ND) virus, reared under heat stress condition. A total of 420, one day-old broiler chicks were randomly divided into 4 groups and kept under elevated summer temperature and treated with four levels of added ascorbic acid i.e., 0 (control group), 200, 400, 600 mg/kg in diets for 28 days, from 21 to 49 days of experimental period. HI antibody titer and ELISA antibody titer against ND virus were significantly ($P<0.05$) higher at 600 mg/kg Vitamin C in the diet than the others treatments. Apparently, adding ascorbic acid at 600 mg/kg in the diet could improve humoral immunity in broilers reared under heat stress.

Keywords: Ascorbic acid, Broiler, Immunity

Introduction

Broiler chickens are homoeothermic animals, which mean that they maintain their central body temperature within a slight range irrespective of ambient temperature. So, birds have a thermoneutral zone that should be described as being a range in the environmental temperature in which energy needs for thermoregulation is minimum and the net energy for production is maximum (Furlan, 2002). Various nutritional strategies have been suggested to overcome the negative effect of heat stress (HS) in broilers (Austic, 1985; Leeson, 1986; Teeter and Belay, 1996; Yahav, 2000) since poultry synthesise vitamin C endogenously, neither domestic nor foreign standards of poultry nutrition quote bird requirements for this vitamin (Larbier and Leclercq, 1995; Smulikowskiej, 1996). However, numerous references can be found in literature (Pardue and Thaxton, 1986; Lechowski and Nagórna-Stasiak, 1995; Kontecka, 1997; Zulkifli et al., 2000a&b) reporting a favourable influence of the addition of vitamin C either in drinking water or in feed on poultry production. Several studies have shown that the environmental temperature may influence the immune response of poultry (Henken, 1982; Beard and Mitchell, 1987; Donker et al. 1990; Tayeb et al., 2011). The mechanism by which the environmental temperature may act as an immune suppressor is not fully understood. However, it is speculated that the

increased activity of the adrenal gland due to stress increases the level of serum corticosteroids, which cause suppression of cell proliferation factor, or interleukin II (Siegel and Latimer, 1984). The intensity and the duration of the stress induced by temperature may impair the immune system of poultry (Austic, 1985; Barbour et al., 2010); however, the effect of the environmental temperature depends on the degree of habituation of the bird at the time of immunization. (Arjona et al., 1990) observed that environmental temperature stress early in life may induce resistance to weather variation in adult animals.

The current study evaluated the effect of high environmental temperature on the humoral immune response of broilers vaccinated against Newcastle disease virus (NDV).

Materials and Methods

A total of one hundred fourteen, day-old broiler chickens (Rose commercial line) were reared in battery brooders located in the poultry farm of the Faculty of Agriculture and Forestry, University of Duhok, Iraq. They were fed with commercial starter and then finisher broiler feed and kept on continuous light and water supply *ad libitum* from the day of hatch till the termination of the experiment. At 21 days of age, all birds were divided into 4 groups (35 birds per group).

Birds were exposed to heat stress at $34\pm 1^{\circ}\text{C}$ and $65\pm 5\%$ relative humidity for eight consecutive hours per day beginning at 21 days of age till 49 days of age. One group was kept as control without supplementation of any vitamin C. Birds of 2nd group were supplemented with 200mg/kg Vit. C Third group was supplemented with 400mg/kg Vit. C Fourth group was supplemented with 600mg/kg Vit. C Birds of control and experimental groups were vaccinated against ND include using LaSota vaccine at 7, 18, 28, 38 days of age. On 21, 31, 41 and 49 days of age, blood samples from 10 randomly selected birds per experimental group were collected for ELISA and HI test. HI test was performed as described by Gould and Clegg (1987) and ELISA test was assayed according to the method designed by William et al. (1997).

Statistical analysis

Results are expressed as means \pm SEM for each group. Groups were tested for differences by Duncan protected significant differences test, using the CRD. Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

The geometric means of HI titer against ND vaccine was significantly reduced in heat stressed control group (G1) on day 31, 41 and 49 of age in comparison to the other treated groups as shown in table (1). The result of the present study is in agreement with those of Zulkifi et al. (2000a), Amedy (2008) and Barbour et al. (2010), who showed that heat stress cause a reduction in antibody synthesis. Whereas, heat-stressed birds treated with 600 mg/kg Vit. C showed significant elevation of HI titer in comparison to the other treated groups (G2, G3, G4) on 31, 41 and 49 day of age. This result agrees with the findings of Aengwanich et al. (2003), who reported that humoral immunity in broilers under chronic heat stress is Vit. C dose-dependent. Moreover, the heat stressed birds

treated with 400mg/kg Vit. C in the diet showed significant elevation of HI titer at all the days of this study compared with the heat stressed birds treated with 200 mg/kg Vit. C supplement. Poultry have the ability to synthesize ascorbic acid, but this ability was inadequate under high environmental temperature (Roussan et al., 2008; Barbour et al., 2010).

In the present study, the significant difference at the level of HI titer between the same group but at the different period of the study, in both control group (G1) and group 2 (treated with 200 mg/kg Vit. C in the diet) and group 4 showed a significant reduction of HI titer at the period of 41 days of chickens age in comparison to the period of 31 days and 49 days of chickens age as shown in table 2. Whereas, the HI titer of the group 3(400 mg/kg Vit.C. in the diet) showed reduction of HI titer on day 49 (Table 2). This result is in agreement with the finding of Thaxton and Siegel 1970, and Amedy (2008) who concluded that the influence of environmental temperature variations on the immune response depends on the degree of the adaptation of the chickens at the time of immunization.

The results of ELISA antibody test of the experimental groups showed that heat stressed birds not treated with Vit.C. experienced a significant reduction in ELISA titer (Table 3). However, ELISA titer of ND at the rate of 600 mg/kg Vit. C in the diets was significantly higher than other. Significant reduction of ELISA titer in the diet of G1, G2 and G4 on day 41 in comparison of 49 day in G3 was observed (Table 4). Such result was also reported by Aen wanich et al. (2002) who concluded that that ascorbic acid could improve HI and ELISA titer of ND. Similarly Gross (1992) and Amedy (2008) reported that ascorbic acid could improve immune response in birds under stress and disease condition. The possibility of increasing antibody titer to vaccination in the Vit.C supplemented chickens may be due to speeding up of differentiation of lymphoid organs by increasing the activity of hexose monophosphate pathway thus increasing circulating antibodies (Dieter and Breitenbach, 1971).

Table 1: Geometric mean of HI titer against ND in heat stressed broiler chickens of control & treated groups

Treat	31 days	41 days	49 days
Control (G1)	130.800 ± 5.032^d	102.400 ± 3.370^d	114.500 ± 3.780^d
Group 2(G2)	153.600 ± 5.938^c	120.600 ± 5.880^c	136.600 ± 1.939^c
Group 3(G3)	231.600 ± 7.259^b	195.400 ± 5.121^b	157.500 ± 6.354^b
Group 4(G4)	296.00 ± 3.932^a	224.200 ± 2.215^a	269.600 ± 7.332^a

^{a-d} Mean values bearing different superscript differ significantly ($P < 0.05$)

Table 2: Geometric mean of HI titer against ND in heat stressed broiler chickens at different days

Chickens age	Control group (G1)	Group 2 (G2)	Group 3 (G3)	Group 4 (G4)
31 days	130.800 ± 5.032^a	153.600 ± 5.938^a	231.600 ± 7.259^a	296.000 ± 3.932^a
41 days	102.400 ± 3.370^c	120.600 ± 5.880^c	195.400 ± 5.121^b	224.200 ± 2.215^c
49 days	114.500 ± 3.780^b	136.600 ± 1.939^b	157.500 ± 6.354^c	269.600 ± 7.332^b

^{a-c} Mean values bearing different superscript differ significantly ($P < 0.05$)

Table 3: Mean ELISA titer of ND in the heat stressed broiler chicks of control and treated group

Treat	31 days	41 days	49 days
Control (G1)	1377.090 ± 44.019 ^d	954.890 ± 19.743 ^d	1024.760 ± 25.618 ^d
Group 2(G2)	2510.380 ± 20.605 ^c	1906.850 ± 39.053 ^c	2234.210 ± 10.808 ^c
Group 3(G3)	2855.880 ± 17.069 ^b	2459.960 ± 110.654 ^b	2538.850 ± 49.553 ^b
Group 4(G4)	3419.090 ± 17.249 ^a	2983.340 ± 27.600 ^a	3436.970 ± 46.665 ^a

^{a-d} Mean values bearing different superscript differ significantly (P<0.05)

Table 4: Mean of ELISA titer of ND in heat stressed broiler chickens at different days

Chickens age	Control group (G1)	Group 2 (G2)	Group 3 (G3)	Group 4 (G4)
31 days	1377.090 ± 44.019 ^a	2510.380 ± 20.605 ^a	2855.880 ± 17.069 ^a	3419.090 ± 17.249 ^a
41 days	954.890 ± 19.743 ^b	1906.850 ± 39.053 ^c	2459.960 ± 110.654 ^b	2983.340 ± 27.600 ^b
49 days	1024.760 ± 25.618 ^c	2234.210 ± 10.808 ^b	2538.550 ± 49.553 ^b	3436.970 ± 46.665 ^a

^{a-c} Mean values bearing different superscript differ significantly (P<0.05)

Conclusion

The ascorbic acid supplementation at 600mg/kg in the diet of broiler chicks will induce some beneficial effects for broiler during heat stress especially in the summer in hot climate area to improve the performance and immunity.

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