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Effect of Dietary Ascorbic Acid on Performance and Immune Response of Heat Stressed Broiler Chicks

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Abstract: A total of 112, day-old broiler chicks were reared under summer temperature of the Sudan (35-45°C) to study the effect of supplementation of ascorbic acid on the feed intake, Feed Conversion Ratio (FCR), weight gain, immune status and ratio of weight of bursa, thymus and spleen to body weight. Four level of ascorbic acid 0 (control), 150 (low level), 350 (moderate level) and 550 (high level) mg/kg were used from day 14-42 day. Birds fed low and moderate levels of ascorbic acid (150, 350 mg/kg) show high feed intake compared to those fed high level (550 mg/kg), which resulted in improvement in body weight and feed conversion ratio of the moderate and low levels. Dressing percentage was not affected by different level of ascorbic acid. Total white blood cells were reduced as the level of ascorbic acid was increased. Lymphocytes increased in birds fed moderate or high levels of ascorbic acid. Heterophils were decreased in the group fed the moderate level and high levels of ascorbic acid. Monocytes decreased in the groups fed the high level, However, basophils and esinophils were not affected by the different levels of ascorbic acid. The mean weight of lymphoid organs or the ratio of lymphoid organs to body weight was not affected by the different treatments. Antibody titer against Newcastle virus was increased in birds fed diets supplemented with different levels of ascorbic acid.

Key words: Ascorbic acid, feed intake, lymphoid organs

INTRODUCTION

The domestic fowl is a homeothermic which can live comfortably only in a relatively very narrow zone of thermo-neutrality ranged from 18-22°C (El-Husseiny and Creger, 1981). One of the problems challenging poultry industry is the high ambient temperature which persists for almost five months of the year (March to July) in most agro-ecological zones in Sudan. High environmental temperatures have deleterious effects on poultry, reducing growth rate, feed intake, live weight gain and feed efficiency, digestibility of nutrients, egg production, egg weight, haugh unit and yolk index (Mills *et al*., 1999) mortality and immunity (Naseem *et al*., 2005). The physiological functions of broiler are affected also by heat stress (El-Husseiny and Creger, 1981). Supplementation of ascorbic acid had been reported to improve heat resistance, protect the immune biological tissues in growing birds and reduced their mortality to infection associated with elevated ambient temperatures (Pardue *et al*., 1984). The addition of ascorbic acid to the diet improves the immune response of birds during heat stress (Zahraa, 2008). The different cells of leukocyte of broiler are affected by high temperature (Borges *et al*., 2004).

The purpose of the present study was to evaluate the effect of various level of dietary ascorbic acid on growth performance, antibody titer and white blood cells of broiler chicks under high temperature.

MATERIALS AND METHODS

One hundred and twelve day-old broiler chicks (hubbared) were reared in an open sided poultry house in the faculty of Animal Production University of Khartoum under diurnal cyclic temperature. The temperature of the poultry house was recorded 3 time/day (at 7am, 3pm and 7pm) using thermometer which range from (35-45°C). The birds were divided into four groups, designated as A, B, C and D containing 28 birds each, on the 14th day of age. Group A acted as control fed diet without ascorbic acid supplementation, group B C and D were supplemented with 150, 350 and 550 mg/kg ascorbic acid, respectively. The ingredient composition of the experimental diets is shown in Table 1. The determined chemical analysis of the experimental diets and their calculated analysis are shown in Table 2. All the birds were vaccinated on days 28 against Newcastle Disease (ND) virus (Lasota strain intraocular). Feed intake, Feed Conversion Ratio (FCR) and body weight was calculated weekly.

Blood samples were collected on day 42, three birds from each pen were used (12 sample/treatment). The birds were bled from the wing vein using sterile disposable syringe. The area of collection was scrubbed by disinfectant (70% alcohol) before the wing vein was punctured. Four ml of blood was collected from each sample. The blood was discharged immediately into collecting tubes 2 ml tube with anticoagulant (Lethem

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Table 1: The ingredient composition of the experimental diets (%)

Ingredients (%)	Experimental diets
Sorghum	63.5
Groundnut cake	19.0
Sesame cake	10.0
Wheat bran	1.5
Super concentrate*	5.0
Di Calcium	0.5
Lime stone	0.2
Common salt	0.3
Total	100.0

*Super concentrate contains the following/kilogram: crude protein 40%, metabolizable energy 2100 kcal/kg, ether extract 2%, crude fiber 2%, calcium 10%, Av. Phosphorus 4%, Lysine 12%, Methionine 3%, Methionine+cystine 3.2%. Diet 1 = 0 mg/kg vitamin C (control), Diet 2 = 150 mg/kg vitamin C, Diet 2 = 350 mg/kg vitamin C, Diet 2 = 550 mg/kg vitamin C

Table 2: Calculated analysis of the experimental diets (%)

Item	NRC (1994)	EBD
Metabolizable energy (kcal/kg)	3200.00	3114.00
Crude protein (N% 6.25)	21.50	22.10
Lysine	1.10	1.08
Methionine	0.50	0.46
Calcium	1.00	1.30
Av. Phosphorus	0.45	0.42

EBD = Experimental Basal Diets

Table 2B: Determined analysis

Item	Experimental basal diets
Dry mater	93.07
Crude protein	23.10
Crude fiber	4.53
Ash	5.06
Ether extract	4.62
Metabolizable energy (kcal/kg)	3114.00

heparin) for blood cell examination (total white blood cells, lymphocytes, heterphils, monocytes, basophils, esinophils). The other 2 ml was collected in tube without anticoagulant for serum separation for antibody titer evaluation. The serum was carefully collected into small sterile vials and kept at -20°C until used.

Antibody titers against Newcastle virus were determined by ELISA by using a ProFlock antibody test kit 9 according to the manufacturer's directions for calculation of result, an S/P ratio.

At the end of each experiment, birds were slaughtered and their lymphatic organs (spleen, bursa and thymus) weight were determined and the spleen/body weight, bursa/body weight and thymus/body weight ratios were calculated on day 42 (Lecui *et al*., 1998).

Data were analyzed using repeated measurement of the ANOVA procedure of Statistical Analysis System (SAS,9). Duncan Multiple Range Test (DMRT) was used to determine the significance between means.

RESULTS

The overall performance of broiler chicks fed diet supplemented with different level of ascorbic acid during summer is summarized in Table 3. The group fed diet supplemented with 150, 350 mg/kg ascorbic acid showed significantly ($p \leq 0.05$) higher weight gain. However, the group fed diet with 0.550 mg/kg ascorbic acid had lower weight gain ($p \leq 0.05$). There was significantly ($p \leq 0.05$) low feed conversion ratio in bird fed diet supplemented with 350 mg/kg of ascorbic acid compared to those fed 150, 550 mg/kg. There was a significant ($p \leq 0.05$) effect of different levels of vitamin C in feed intake of different group of bird. Significant ($p \leq 0.05$) low feed intake was observed in birds fed diet supplemented with 0 mg/kg followed by 550 mg/kg ascorbic acid.

There was no significant ($p \geq 0.05$) in dressing percentage of the different experimental groups as shown in Table 3.

The effect of dietary supplementation of ascorbic acid on differential leukocyte count is shown in Table 4. There

Table 3: Overall performance of broiler chicks fed different levels of ascorbic acid under summer condition

Parameter	0	150	350	550	SEM
Total weight gain	1132.80^{b}	1216.50^{a}	1263.00^{a}	1074.50^{b}	72.617**
Total feed intake	2447.00^{b}	2590.50^{ab}	2639.50^{a}	2530.50^{ab}	58.627**
Total feed conversion ratio	2.161^{ab}	2.130^{ab}	2.089^{b}	2.355^{a}	00.100**
Live body weight	1176.25^{b}	1260.12^{a}	1307.75^{a}	1118.50^{b}	58.754**
Dressing (%)	0069.96	70.065	70.108	71.100	0.93NS

Means in the same row with the same letter are not significantly different ($p \leq 0.05$)

Table 4: Blood analysis for total white blood cells and differential leukocyte count of broilers fed different levels of ascorbic acid

Treatment parameters						
LOAA (mg/kg)	TWBC	Lymphocyte (%)	Heterophil (%)	Monocytes (%)	Esinophil (%)	Basophil (%)
0	2.1	54.125^{b}	29.250^{a}	7.500^{a}	12.125	4.125
150	2.0	54.375^{b}	28.750^{a}	6.500^{ab}	9.750	4.750
350	2.0	61.750^{a}	23.125^{b}	5.250^{b}	11.625	3.375
550	1.9	62.375^{a}	23.625^{b}	4.875^{b}	13.500	3.750
SEM	0.793NS	1.443**	0.886**	0.614**	1.411NS	0.499NS

Means in the same row with the same letter are not significantly different ($p \leq 0.05$). LOAA = Levels of Ascorbic Acid (mg/kg), TWBC = Total White Blood Cells

Table 5: Effect of different levels of ascorbic acid on lymphoid organs of broilers (g/bird)

	Bursa	Thymus	Spleen	BBWR	TBWR	SBWR
0	0.8313±0.37	3.5363±1.03	0.8463±0.37	0.069±0.03	0.29±0.08	0.07±0.03
150	0.6125±0.17	3.3375±1.59	0.6775±0.27	0.049±0.02	0.29±0.13	0.05±0.02
350	0.7013±0.26	2.8263±1.26	0.6512±0.19	0.050±0.02	0.22±0.10	0.05±0.01
550	0.6075±0.23	3.4613±1.23	0.6125±0.19	0.050±0.02	0.29±0.10	0.05±0.02

Means in the same row with the same letter are not significantly different ($p \leq 0.05$). BBWR = Bursa/body weight, TBWR = Thymus/body weight, SBWR = Spleen/body weight

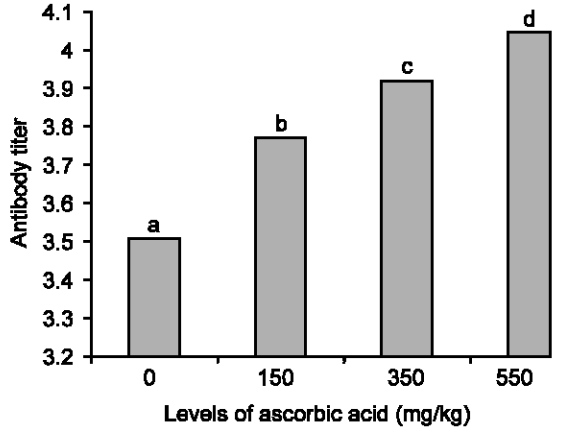

Fig. 1: Geometric mean titers against NDV* (expressed as log10) of broiler chicks at 42 day of age. *Newcastle disease virus

were no significant different ($p \geq 0.05$) in the total white blood cell count between the different groups of birds. However, there was a numerical reduction in the number of total white blood cell when the different levels of ascorbic acid were supplemented to the basal diet.

The percentage of lymphocytes was increased significantly ($p \leq 0.05$) in birds fed diet with moderate level (350 mg/kg) or high (550 mg/kg) level of ascorbic acid compared to those fed 0 and 150 mg/kg ascorbic acid. The percentage of Heterophil was decreased significantly ($p \leq 0.05$) in group fed moderate level (350 mg/kg) or high level (550 mg/kg) of ascorbic acid. Moreover, the results indicated that the high level (550 mg/kg) of ascorbic acid resulted in a decreased in the number of Monocytes but there were no significant different ($p \leq 0.05$) in the number of Basophil and Esinophil between the different experimental groups.

There was no significant different ($p \geq 0.05$) in the mean weight of lymphoid organs or the ratio of lymphoid organs to body weight between the different treatments (Table 5).

Geometric mean titers against Newcastle disease virus of broiler chicks are presented in Fig. 1. The result indicate that antibody titer against Newcastle disease virus was significantly ($p \leq 0.05$) increased in bird fed diet supplemented with different level of ascorbic acid compared to those fed un supplemented diet.

DISCUSSION

The results of this study revealed that there was an increase in the body weight of birds fed diet supplemented with low (150mg/kg) or moderate (350 mg/kg) level of ascorbic acid compared to those fed non supplemented diet Table 3. This finding in agreement with Farooqi *et al*. (2005); Lecui *et al*. (1998) and Raja and Qureshi (2000) who observed an increase in the body weight of chicken fed diet supplemented with Vitamin C during acute heat stress.

During this experiment it was observed that the feed intake was improved with vitamin C supplementation at low (150 mg/kg) and moderate (350 mg/kg) level. This results supported by Farooqi *et al*. (2005) who observed better results when the diet was supplemented with vitamin C. This result was not in agreement with (Blaha and Kroesna (1997), Jaffar and Blaha (1996) who reported, that feed intake of broilers was not affected by the supplementation of Vitamin C. This may be explained by the differences in the condition of the experiments.

The results obtained in this experiment demonstrated a better FCR with vitamin C supplementation. Better FCR was observed in the groups fed 350 mg/kg of vitamin C. This result was close to the result obtained by Blaha and Kroesna (1997); Mckee *et al*. (1997); Anwar *et al*. (2004) whom also detected an improvement in FCR of broilers as a result of Vitamin C supplementation during heat stress.

The effect of different level of ascorbic acid on live body weight was significantly enhanced by the low level (150 mg/kg) and the moderate level (350 mg/kg) of vitamin C. This finding in agreement with the finding of Kafri and Cherry (1984); Mckee *et al*. (1997) who reported that under heat stress conditions dietary supplemented with ascorbic acid alleviates the effect of heat stress on the performance of broiler chicks. Conversely, high level (550 mg/kg) of vitamin C resulted in lower live body weight. This result agreed with the results obtained by (Sabah *et al*., 2008) who found that addition of Vitamin C at higher doses (500 mg and 750 mg/kg) gave lower performance even when compared with the control (zero Vitamin C level).

Generally, normal total leukocyte counts in chickens (*Gallus gallus* domesticus) were 12000-30000 cell/µl (Jain, 1993). The results revealed that there were no significant different in total white blood cell when the vitamin C was supplemented (Table 4). This result supported by the result obtained by (Farooqi *et al*., 2005)

who reported that addition of ascorbic acid had no effect on white blood cells and differential counts.

The analysis of blood serum samples for differential leukocyte count showed that the percentage of lymphocytes was increased significantly but the percentage of heterophils and monocytes was decreased and there was no significant different in the number of basophil and esinophil in birds fed diet supplemented with vitamin C. These finding were in line with the finding of Wang *et al.* (2001). The decrease in Heterophil percentage could be explained by inflammation as suggested by Ritchie *et al.* (1994). The results obtained are in contradiction with (Aengwanich *et al.*, 2003) who showed no significant difference in the number of Lymphocytes. The discrepancies between these results can be attributed to the difference in experimental procedure.

The obtained results indicate that there was no differences in the weight of lymphoid organs (Table 5). There was no structural changes were observed in shape of bursa or the spleen of any group. This result in agreement with result of (Anwar *et al.*, 2004) who found that ascorbic acid and acetylsalicylic acid (Sb-Asper-C) supplementation during heat stress had no beneficial effects on ratio of weight of bursa, thymus and spleen to body weight of heat-stressed birds.

This study revealed that the antibody titer of broiler chicks produced against Newcastle disease virus was affected with ascorbic acid supplementation (Fig. 1). There was an increase in the antibody titer in groups fed supplemented diet with ascorbic acid. These finding are similar to those of (Tuekam *et al.*, 1994) who observed a positive correlation between antibody titer and ascorbic acid supplementation. Also (Aengwanich *et al.*, 2003) who found an improvement in antibody titer of Newcastle disease of broiler when ascorbic acid was supplemented to the diet.

Conclusion: This study indicated that ascorbic acid supplementation during heat stress had beneficial effects on weight gain, feed conversion ratio and serum antibody development of heat-stressed birds. It is concluded from this experiment that ascorbic acid can be used to minimize susceptibility of the bird to viral disease since it increase antibody titer against Newcastle virus. It can be recommended that vitamin C can be used during high temperature as feed additives with level less than 500 mg/kg to alleviate the negative effect of high temperature.

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