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Effect of Natural Vitamin C on Performance and Certain Haemato-Biochemical Values in Broiler Chickens Exposed to Heat Stress

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AMA, ANA planned the research and conducted the growth trial. EOSH and MHA collected samples and did the lab work. AMA wrote the manuscript.

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Ascorbic acid, Blood hemato-biochemical parameters, Broilers, Performance, Heat stress.

ABSTRACT

During hot climates, the supplementation of extra electrolytes or vitamins such as ascorbic acid (vitamin C) to the drinking water or feed of poultry has become a common practice. Broilers are more susceptible to heat stress as compared to other animals. The current experiment was conducted to examine the effect of commercial vitamin C supplement (VC100, 200 mg/kg), on performance of broilers. Two levels of environmental temperatures: normal (22°C) and high (32°C) and two levels of vitamin C in drinking water (with and without) were arranged in a factorial arrangement resulted in four dietary treatments for the period from 15 to 30 days of age. Results revealed a significant two way interaction for body weight gain (BW) and feed intake (FI) ($P<0.01$) for the first week of the trial (15-22 day). The effect of treatment in the second week (23-30 day) was less pronounced and treatment had no effect on performance. Cumulative performance results for the period (15-30 day) showed that feed intake was affected by temperature ($P<0.05$). It can be concluded that natural vitamin C 100 has no a major impact on cumulative performance or plasma mineral status.

INTRODUCTION

Heat stress is one of the most important factors harmfully affecting overall poultry production (Khan *et al*., 2011, 2014). High temperature and humidity exert severe stress on birds leading to reduced performance (Khan *et al*., 2012; Chand *et al*., 2014). Broiler performs well within a fairly wide range of temperatures (18 and 22°C). However, this wide range thermoneutral temperature is not ideal for the ideal feed efficiency. For example, Kampen (1984) reported that the maximum growth rate of broilers occurs in the range of 10-22° C while the ideal feed efficiency occurs at 27°C. Charles (2002) on the other hand, reported a lower range of temperature for optimum performance in broilers (18-22°C). At temperatures above 30°C, broilers reduce feed intake and as a result gain less per unit of feed (Chand *et al*., 2016). It is generally agreed that heat stress in broilers reduces feed intake, body weight, immune status and increase mortality (Chand *et al*., 2017).

Poultry are renal synthesizers of ascorbic acid (vitamin C) (Maurice *et al*., 2002) and diets are not normally fortified; hence, no recommended requirement is established by the NRC (1994). The endogenous production of vitamin C synthesis is usually considered not sufficient for the biological demands in poultry, especially during the severe environmental conditions (Pardue and Thaxton, 1986). It was concluded that during certain conditions, vitamin C supplementation provides benefit to poultry (Pardue and Thaxton, 1986). Significant improvements in growth of chicks were reported when vitamin C was supplemented for stressed birds. Heat-stressed broilers fed vitamin C supplemented diets consumed more feed, were less stressed, and had reduced body temperature and respiratory rates than control birds (Kassim and Norziha, 1995). Accordingly, the objective of the present study was to evaluated the efficacy of natural vitamin C, a naturally occuring vitamin C, on broiler performance and blood hematoogy under moderate heat stress for the period from 15 to 30 days of age.

MATERIALS AND METHODS

The current study was approved by the ethical committee on right and welfare of animal, King Saud University, Saudi Arabia.

The current study was conducted by utilizing 144 15-days old unsexed Ross 308 broiler chicks obtained from a commercial hatchery. Chicks were allotted to 24

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cages in a 4 deck cage system to construct 6 replicates per treatment and received control diet (Table I). The basal feed contained 3000 kcal/kg metabilizable energy and 18.5% crude protein. Each cage had breadth of 3000 cm^2 (50 cm length, 60 cm width and 36 cm depth). Broilers were randomly assigned to 4 treatments in 2x2 factorial arrangements: T1, control; T2, birds were subjected to heat stress at 32°C; T3, water was supplemented with 200 mg VC-100/L; and T4, water was supplemented with 200 mg VC-100/L + birds were subjected to heat stress at 32°C. The vitamin C used in this trial is a natural product derived from Amla which is obtained from the plant *Emblica officinalis*.

The chicks were provided feed and water ad libitum, while light schedule was maintained for 24 h. Chicks were reared in standard brooders and vaccinated against Marek's disease, Newcastle and Infectious Bronchitis. All birds were provided a diet based on corn and soybean in mash form (Table I).

During the finisher period (23-30 days), body weight (BW) and feed intake (FI) were recorded weekly for each pen, and feed conversion ratio (FCR) was determined from the given data of FI and BW. Mortality was recoded if occured (Abudabos *et al*., 2017).

On day 30, blood samples were collected from three birds from each treatment with or without EDTA. Blood samples with EDTA were analyzed for white blood cell counts (WBC), total red blood cell counts (RBC), hemoglobin content (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), standard deviation in red cell distribution width (RDW-SD), platelet count (PLT), mean platelet volume (MPV), coefficient variation of red cell distribution width (RDW-CV), platelet distribution width (PDW) hematocrit (HCT) and plateletcrit (PCT). Serum was separated by centrifuging plain tubes at 5ºC and 3000 rpm for 10 min. Thereafter, sera were transferred into eppendorf tubes and stored at -20ºC until further analysis. Serum sodium, chloride, magnesium, total calcium and phosphorus were determined using commercial kits (M di Europa GmbH Wittekamp 30. D-30163 Hannover, Germany).

Table I. Feed composition.

Ingredients	%
Yellow corn	61.94
Soybean meal	31.00
Palm oil	3.00
Dicalcium phosphate	2.20
Ground limestone	0.61
Choline chloride	0.10
DL-methionine	0.20
L-lysine	0.15
Salt	0.30
Vitamin-mineral premix[1]	0.50
Calculated analysis	
ME, kcal/kg	3000
Crude protein, %	18.5
Non phytate P, %	0.40
Calcium, %	1.00
Lysine, %	1.00
Methionine, %	0.45

[1]Vitamin-mineral premix contains in the following per kg: vitamin A, 2400000 IU; vitamin D, 1000000 IU; vitamin E, 16000 IU; vitamin K, 800 mg; vitamin B1, 600 mg; vitamin B_2, 1600 mg; vitamin B_6, 1000 mg; vitamin B_{12}, 6 mg; niacin, 8000 mg; folic acid, 400 mg; pantothenic acid, 3000 mg; biotin 40 mg; antioxidant, 3000 mg; cobalt, 80 mg; copper, 2000 mg; iodine, 400; iron, 1200 mg; manganese, 18000 mg; selenium, 60 mg, and zinc, 14000 mg.

Table II.- Body weight, feed intake and feed conversion ratio of broiler chickens given experimental treatments for two weeks.

Experimental period	Parameters	Vitamin C (0 mg/L)		Vitamin C (200 mg/L)		SEM	Statistical probabilities		
		22°C	32°C	22°C	32°C		Temp.	Vit. C	Temp. × Vit. C
Vitamin C for one week (15-22 days)	Body weight (g)	445.2	400.4	426.9	453.2	11.9	NS	NS	**
	Feed intake (g)	617.9	565.3	582.7	613.8	12.4	NS	NS	**
	FCR	1.389	1.415	1.367	1.356	0.02	NS	NS	NS
Vitamin C for second week (23-30 days)	Body weight (g)	558.1	544.4	569.2	532.5	15.0	NS	NS	NS
	Feed intake (g)	878.9	860.7	882.0	825.4	15.7	NS	NS	NS
	FCR	1.575	1.585	1.553	1.554	0.031	NS	NS	NS

*P<0.05; **P<0.01; NS, not significant; SEM, standard error of the mean; FCR, feed conversion ratio; Temp., temperature.

Table III.- Cumulative performance of broiler chickens given experimental treatments from 15 to 30 days.

Parameters	Vitamin C (0 mg/L)		Vitamin C (200 mg/L)		SEM	Statistical probabilities		
	22°C	32°C	22°C	32°C		Temp.	Vit. C	Temp. × Vit. C
Body weight (g)	1003.3	944.7	996.1	985.6	20.6	NS	NS	NS
Feed intake (g)	1496.9	1425.9	1464.7	1439.2	21.9	*	NS	NS
FCR	1.49	1.51	1.47	1.46	0.02	NS	NS	NS

*P<0.05; NS, not significant; SEM, standard error of the mean; FCR, feed conversion ratio; Temp., temperature.

Statistical analysis

All statistical analysis was performed using the Statistical Analysis System (SAS, 2009) for randomized complete block design with 2 x 2 factorial arrangements of treatments, in which each treatment was assigned to 6 replicate pens. The data were tested for main effects of vitamin C, temperature (temp.) and for interaction effect for vitamin C x Temp. Statistical significance was assessed at (P<0.05).

RESULTS

Growth performance, feed intake and feed conversion results for the periods (15-22 d) and (23 to 30 d) are shown in Table II. For the first week (15-22 d), a two way interaction was significant for BW and FI (P<0.01). When the temperature increased from 22 to 32°C, the gain decreased when no vitamin C was supplemented into drinking water from 445.2 to 400.4 g. However, BW increased when vitamin C was supplemented at the rate of 200 mg/l at the high temperature (32°C). In the absence of vitamin C supplementation at 22°C, birds consumed more feed as compared to birds which had been subjected to 32°C (617.9 *vs.* 563.3 g, respectively). In the contrary, when vitamin C was supplemented to the water, birds which were subjected to the high temperature (32°C), consumed more feed. The interaction of temperature and vitamin was significant (P<0.01) for body weight and feed intake.

This result is in agreement with McKee and Harrison (1995) who reported that under heat stress conditions, broilers consumed more feed when vitamin C was supplemented.

No significant differences in feed conversion ratios were found due to treatment (P>0.05). For the second week (23-30 d), treatments had no effect on BW or FC; however, FI was affected by temperature. Birds on the high temperature (32°C) decreased their feed intake by 37 g as compared to the other group (P<0.05).

Cumulative performance results for the period (15-30 d) are shown in Table III. Neither temperature nor vitamin C had an effect on BW or FC; however, FI was affected by temperature (P<0.05). Birds on the high temperature (32°C) decreased their feed intake by 50 g as compared to the normal temperature group (P<0.05).

Table IV.- Effect of different treatments on blood of broilers at 30 day of age.

	Treatment					
	T1	T2	T3	T4	SEM	P
Hematology						
WBC (×10³/µL)	116.2	119.0	111.3	110.0	±3.9	NS
RBC (×10⁶//µL)	3.5	3.5	3.8	3.9	±0.4	NS
Hb (%)	36.1	32.8	34.1	36.1	±1.4	NS
MCV (fL)	103.9	114.9	114.9	115.0	±3.6	NS
MCH (pg)	116.6	93.3	88.9	93.9	±14.7	NS
MCHC (gHb/100 ml)	117.0	81.2	77.4	81.7	±19.0	NS
RDW-CV (%)	10.7	9.4	9.9	10.1	±0.4	NS
RDW-CD (fL)	44.0	43.3	45.7	49.7	±1.2	NS
HCT (%)	36.8	40.3	43.9	44.2	±4.7	NS
PLT (×10³/µL)	130.3	72.7	105.0	98.7	±34.8	NS
MPV (fL)	11.8	10.9	11.8	11.6	±0.3	NS
PDW	16.1	11.5	13.6	14.1	±1.4	NS
PCT (%)	0.15	0.08	0.12	0.11	±0.04	NS
Mineral concentration						
Na (mmol/l)	13.7	10.0	7.7	8.7	±3.2	NS
Cl (mmol/l)	100.7	99.0	97.3	95.0	±2.1	NS
Mg (mmol/l)	2.3	2.4	2.6	2.3	±0.1	NS
Ca (mg/ dl)	9.1	10.5	9.3	9.7	±1.1	NS
P (mg/ dl)	11.4	9.3	10.4	10.4	±0.9	NS

Ca, calcium; Cl, chloride; HCT, hematocrit; HGB, hemoglobin content; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; Mg, magnesium; Na, sodium; P, phosphorus; PCT, plateletcrit; PDW, platelet distribution width; PLT, platelet count; RBC, total red blood cell counts; RDW-CV, coefficient variation of red cell distribution width; RDW-SD, standard deviation in red cell distribution width; WBC, white blood cell counts.

Table IV shows the effect of treatment on some hematological and biochemical parameters of broiler blood profile. The values of the blood hematological parameters measured in this trial were comparable and treatment had no effect on blood hematological values. However, serum mineral concentrations were affected by

temperature. In this trial, lower Na, Cl and P levels were found in plasma at high temperature group as compared to normal temperature group (8.5 *vs.* 14.3 mmol/l Na; 95.0 *vs.* 99.0 mmol/l Cl; and 9.5 *vs.* 11.3 mg/dl P, respectively).

DISCUSSION

Heat stress depresses feed intake and growth performance in broiler. Reduction in feed intake decreases linearly with increasing temperature. Reduction in feed intake is the first response of the heat stress in broiler. Reduction in feed consumption is proportional to low consumption of ascorbic acid. Supplementation of ascorbic acid ameliorates the feed intake and growth rate during high ambient temperature. The increased production in the presence of ascorbic acid during heat stress may be due to high oxygen consumption, thyroid activities and feed intake (Khan *et al*., 2012).

At higher environmental temperatures and during heat stress, birds start to pant as a mean of reducing body temperature. Khattak *et al*. (2012) demonstrated that panting leads to disturbances in acid base balance of the bird. The reduction in circulating mineral levels under heat stress has been reported by various researchers. For example, Belay *et al*. (1993) reported poor absorption of Ca, K, and P in turkeys which were subjected to heat stress. Others, showed poor retention and increased excretion of minerals such as Na, K, Ca, Cu and P and in heat stressed chickens (Belay *et al*., 1992).

CONCLUSION

It can be concluded that negative effects on the broiler performance specially feed intake was associated with higher ambient temperatures. The natural vitamin C improved feed intake and weight gain during the first week but not during the second week.

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Statement of conflict of interest

Authors have no potential conflict of interest.

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