

EFFECT OF NATURAL ASCORBIC ACID ON PERFORMANCE AND CERTAIN HAEMATO-BIOCHEMICAL VALUES IN LAYERS EXPOSED TO HEAT STRESS

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ABSTRACT

A four week trial examined the effects of dietary vitamin C supplement (200 mg/kg) and heat stress on performance of layers and blood biochemical profile. Two levels of environmental temperatures: thermoneutral (TN, 22°C) and high temperature (HT, 32°C) and two levels of vitamin C supplement (without and with) were arranged in a factorial arrangement resulted in four dietary treatments on feed intake (FI), egg mass (EM), feed conversion ratio (FCR), egg shell strength (ESS) and blood hematology in one strain of layers (Hy-Line W-36) from 65 to 69 weeks of age. Four treatments were assigned to nine replicates with three hens per pen. Treatment 1 was a corn-soy control in TN, treatment 2 was control in HS, treatment 3 was control + 0.2 g/l ascorbic acid in TN and treatment 4 was control + 0.2 g/l ascorbic acid in HS. Hens which were subjected to TN environment had higher FI as compared to those which had subjected to HS environment ($P < 0.05$). Egg mass was not affected by vitamin C, temperature or their interaction ($P > 0.05$). Feed conversion ratio was affected by temperature but not with Vitamin C supplementation. Hens which were subjected to TN had better FCR as compared to those which were subjected to HS for weeks two, three, four and cumulative period ($P < 0.01$; $P < 0.05$; $P < 0.01$; $P < 0.01$, respectively). Hens which were subjected to HS had weaker shell strength at week two ($P < 0.01$), week four ($P < 0.05$) and cumulative period ($P < 0.001$). On the other hand, there was a significant interaction for vitamin C x Temperature at week three ($P < 0.001$). Vitamin C increased Na and P concentrations in serum ($P < 0.05$, $P < 0.01$, respectively) and decreased Ca and P concentrations as compared to those which were subjected to TN ($P < 0.05$, $P < 0.05$, respectively). It can be concluded that natural vitamin C did not have a major impact on cumulative performance or plasma mineral status.

Key words: Heat stress, thermoneutral, vitamin C, layers Feed conversion ratio (FCR), Hen performance,

INTRODUCTION

Heat stress has a negative impact on economic losses in poultry production both for tropical and temperate areas (Al-Saffar and Rose, 2002; Khan et al., 2011). Heat stress remains a continuous challenge for the layer enterprises during summer season when the area is exposed to recurrent summer heat waves (Khan et al., 2012; Chand et al., 2016). Layers are predominantly susceptible to heat stress because they have to maintain a long production cycle which could extend from 50 to 70 weeks (Mignon-Grasteau et al., 2015).

High environmental temperature, high humidity and low air velocity are factors contribute to heat stress (Balnave and Brake, 2005; Khan et al., 2014a). Heat stress arises when layers struggle in balancing body heat production and body heat loss. Layers depend on several mechanisms to regulate their body temperature within the thermo neutral zone when they are subjected to high environmental temperatures (Simon, 2003). To avoid heat stress, the thermoregulatory mechanisms in poultry are normally stimulated above the thermoneutral zone of 24°C (Celik et al., 2004; Chand et al., 2017). Heat stress

effects become noticeable when temperatures exceed 30°C (Seven, 2008).

Heat imposes severe stress on layers, which leads to poor performance, depresses body weight, egg production, egg weight, shell quality and is generally accompanied by suppression of feed intake (Mashaly et al, 2004; Chand et al., 2014), predisposing birds to various infectious diseases and high mortality rates (Tanor et al., 1984, Maini et al, 2007). Corticosteroid secretion increased in response to stress, corticosteroid has a catabolic effect and cause impairment of cellular functions (Ramnath et al, 2008; Khan et al., 2013a). Also, most of the production energy is diverted to thermoregulatory adaptations during the periods of heat stress which results in oxidative stress induced immunosuppression (Maini et al, 2007).

There are various strategies to minimize the negative effects of heat stress on layer hens by modification of their diets (Ajakaiye et al, 2010). Numerous studies have documented the beneficial effects of vitamin supplementation on egg production and eggshell quality in stressed poultry C (Waseem et al., 2008, Torki et al., 2014). In birds, it was postulated that ascorbic acid stimulates 1,25-dihydroxy-cholecalciferol

and together with it increases calcium mobilization from bones, suggesting that vitamin C has an important role in eggshell formation (Dorr and Balloun, 1976).

The body requirement of ascorbic acid during heat stress in poultry is greater than the amount synthesized by normal tissues and its administration to broilers during heat stress has been shown to be beneficial to the body (Balogun *et al.*, 1996; Khan *et al.*, 2012). Poultry are renal synthesizers of ascorbic acid (AsA) (Maurice *et al.*, 2002) and diets are not normally fortified; hence, no recommended requirement is established by the NRC (NRC, 1994). It is generally assumed that the endogenous synthesis is sufficient to meet biological demands in poultry; however, environmental stressors are known to alter AsA use or synthesis in the poultry (Pardue and Thaxton, 1986; Khan *et al.*, 2014b).

The objective of the current study was to evaluate the efficacy of natural vitamin C on performance and blood hematology of laying hens exposed to cyclic heat stress for the period from 65 to 69 weeks of age.

MATERIALS AND METHOD

Experimental Design and Diets: Two levels of temperature [thermoneutral (TN, 22°C) and high temperature (HT, 32°C)] and two levels of vitamin C supplement (without and with) were applied in a factorial arrangement for a total of four dietary treatments. Birds were kept in 36 pens in two different rooms, with 18 pens per room. One room was assigned a treatment classified as thermoneutral (TN) while the second room was designated as high temperature (HT). High temperature cycle was applied in the HT room. On daily basis, the temperature in the HT room was allowed to cycle between 22°C and 32°C, while temperature in the TN room was maintained at 22°C, throughout the rest of the study. The HT birds were exposed to 32°C from 8:00 to 15:00 then temperature was steadily decreased to 22°C.

All four treatments were fed to one strain of hens (Hy-line) from 65–69 weeks of age for a total time period of four weeks. Each treatment was assigned to 9 replicate pens with 3 hens per pen (412 cm² / hen) for a total of 36 pens. The multi-bird pen was the experimental unit. The study was conducted under a protocol approved by King Saud University and complies with the current laws of Saudi Arabia.

Diets were formulated to meet phase III of the Hy-line strain recommendations (Table 1). Treatment 1 was the control diet (standard corn-soybean meal) and birds were kept in TN environment. Treatment 2 was the control diet and birds were kept in HS environment. Treatments 3 and 4 were supplemented with 0.2 g / l AsA and birds were kept in TN and HS environments, respectively. The supplement used in this experiment

was a commercial vitamin C supplement derived from amla (VC-100).

Measurements

Hen, Egg and Blood parameters: Feed consumption and egg production were measured daily, and egg production calculated on hen day basis. Egg mass was calculated as a factor of egg weight and egg production. All hens were individually weighed every 4 weeks. Feed conversion was calculated as g of feed: g of egg mass.

Blood samples were withdrawn from five randomly selected birds per treatment via brachial venipuncture into EDTA tubes for hematological analysis, and into plain tubes for serological analysis. EDTA tubes were used to analyze various blood parameters such as white blood cell counts (WBC), total red blood cell counts (RBC), hemoglobin content (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), standard deviation in red cell distribution width (RDW-SD), coefficient variation of red cell distribution width (RDW-CV), hematocrit (HCT), platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT). Hematological parameters were determined by methods described by Campbell (1988). Serum was prepared by centrifuging plain tubes at 5 °C and 3000 rpm for 10 min. Thereafter, sera were transferred into eppendorf tubes and stored at -20°C until further analysis. Serum levels of sodium (ion-selective method), chlorine (Labtest method), magnesium (Tonks' method), total calcium (Labtest method) and phosphorus (Basques-Lustosa's method) were determined using commercial kits (M di Europa GmbH Wittekamp 30. D-30163 Hannover, Germany).

Statistical Analysis: Analysis of variance was performed using GLM procedure of SAS (SAS, 2009) for randomized complete block design with 2 x 2 factorial arrangements of treatments, in which each treatment was assigned to 9 replicate pens. The data were tested for main effects of vitamin C (AsA), temperature (temp.) and for interaction effect for AsA x Temp. Statistical significance was assessed at (P<0.05).

RESULTS AND DISCUSSION

Vitamin C supplementation had no significant effect on feed intake during the experiment period (Table 2). However, the results are not consistent with those of Toriki *et al.* (2014) who reported that feed intake improved in layers when they were given 250 mg/kg vitamin C. Temperature had an effect on feed intake for weeks one, two, four as well as at the cumulative period of the experiment ($P < 0.05$). Hens which were subjected to TN environment had higher feed intake as compared to

those subjected to HS (Table 2). Vitamin C and temperature interaction showed no effect on FI ($P > 0.05$). In the present study, heat stress reduced feed intake. These findings confirm earlier studies (Star *et al.*, 2008). It was estimated that feed intake decreased by 1.6% for each one degree temperature rise between 17 and 29°C and the decreased feed intake was explained by a drop in energy requirement rather than an effect of increased temperature *per se*. Daniel and Balnave (1981) reported that heat stress had more effect on feed intake of laying hens as compared to egg production and concluded that hens utilized the feed more efficiently under heat stress.

Egg mass was not affected by vitamin C, temperature or their interaction ($P > 0.05$) (Table 3). These results are in general agreement with the results of Puthongsiriporn *et al.* (2001) who reported similar egg production and egg mass of laying hens which receiving two concentrations of vitamin C (0 to 65 mg/kg diet). The present results disagree with those of Keshavarz (1996), who reported that egg mass increased as a result of supplementing the diets of heat-stressed laying hens with 250 mg/kg vitamin C.

Feed conversion ratio (FCR) was affected by temperature (Table 4). Hens subjected to TN had better FCR as compared to those which were subjected to HS for weeks two, three, four and cumulative period ($P < 0.01$; $P < 0.05$; $P < 0.01$; $P < 0.01$, respectively). Vitamin C supplementation had no effect on FCR. Similarly, Torki *et al.* (2014) reported that 250 mg/kg vitamin C had no impact on FCR in layers.

Egg shell strength was significantly affected by temperature for weeks two, four and the cumulative period (Table 5). Hens which were subjected to HS had weaker shell strength at week two ($P < 0.01$), week four ($P < 0.05$) and cumulative period ($P < 0.001$). The effect of heat stress on egg shell quality has been well documented (Miller and Sunde, 1975; Wolfenson *et al.*, 1979). de Andrade *et al.* (1976, 1977) found that shell quality was not maintained at constant elevated temperatures. A fall in plasma calcium level was speculated to be the cause for the lower shell quality. In the present study, egg shell quality was not maintained during the period of heat stress nor during the two following periods (7 days). It was significantly ($P < 0.05$) affected by both diet and temperature.

Table 6 shows the effect of treatment on mineral concentration. Vitamin C affected Na and P concentrations ($P < 0.05$, $P < 0.01$, respectively). Vitamin C increased Na concentration from 4.3 to 16.1 mmol/l and increased P concentration from 9.1 to 11.4 mg/dl. On the other hand, temperature affected Ca and P concentrations ($P < 0.05$, $P < 0.05$, respectively). Hens which were subjected to HS had lower Ca and P concentrations as compared to those which were subjected to TN (14.0, 19.0 mg/dl for Ca and 9.8, 11.4 mg/dl for P, respectively). Effect of treatment on blood hematology is presented in Table 7. Vitamin C affected only MCH and MCHC ($P < 0.001$, $P < 0.01$).

Table 1. Composition of the basal diets

Ingredient	(%)
Corn	65.8
Soybean meal	15.9
Corn gluten meal	5.9
Corn oil	1.0
Limestone	7.4
Dicalcium phosphate	1.7
Oyster shell	1.5
Salt	0.5
DL-Methionine	0.05
Lysine	0.16
Trace mineral ¹	0.16
Vitamin premix ¹	0.05
Nutrient analysis	
Protein calculated, %	16.5
Protein analyzed, %	16.7
M.E., Kcal/Kg	2860
Calcium calculated, %	4.0
Calcium analyzed, %	3.9
Avail. Phosphorus, %	0.40
TSAA, %	0.66
Lysine, %	0.85

¹ Mineral- vitamin premix provided Mn, 88; Cu, 6.6 mg; Zn, 88 mg; Se, 0.3 mg; vitamin A, 6,600 IU; cholecalciferol, 2,805 IU; vitamin E, 10 IU; vitamin k, 2.0 mg; Riboflavin, 4.4 mg; pantothenic acid, 6.6 mg; niacin 24.2 mg; choline, 110 mg; vitamin B12, 8.8 mg; ethoxyquin, 1.1 mg; per kg diet.

Table 2. Effect of dietary treatments on feed intake of layers exposed to heat stress.

Diet	Vitamin C	Temperature	W1	W2	W3	W4	Cumulative
	(g/l)	(°C)			(g/d)		
1	0	22	114.1	112.1	110.0	110.9	111.8
2	0	32	107.8	101.2	103.0	102.8	103.7
3	0.2	22	118.0	112.3	110.9	114.2	113.9
4	0.2	32	109.4	106.1	106.2	103.5	106.3
SEM		0.1	3.2	3.8	3.1	3.7	2.9

Vitamin C average					
Without	110.9	106.6	106.5	106.9	107.7
With	113.7	109.2	108.6	108.8	110.1
SEM	2.2	2.7	2.2	2.6	2.1
Temperature average					
22 °C	116.1 ^a	112.2 ^a	110.4	112.6 ^a	112.8 ^a
32 °C	108.6 ^b	103.6 ^b	104.6	103.1 ^b	105.0 ^b
SEM	2.2	2.7	2.2	2.6	2.1
Statistical Probabilities					
Vitamin C	NS	NS	NS	NS	NS
Temperature	0.02	0.03	NS	0.018	0.013
Vitamin C x Temperature	NS	NS	NS	NS	NS

Table 3. Effect of dietary treatments on egg mass of layers exposed to heat stress

Diet	Vitamin C (g/l)	Temperature (°C)	W1	W2	W3 (g)	W4	Cumulative
1	0	22	58.3	60.4	58.4	57.9	58.0
2	0	32	59.7	58.7	58.4	54.5	57.0
3	0.2	22	60.8	60.7	59.7	56.2	59.0
4	0.2	32	59.7	59.0	58.6	57.2	58.6
SEM			1.6	1.6	1.1	1.6	1.1
Vitamin C average							
without			59.0	59.6	58.4	56.2	58.3
With			60.3	59.8	59.1	56.7	59.0
SEM			1.1	1.1	0.8	1.1	0.8
Temperature average							
22 °C			59.5	60.5	59.0	57.0	59.0
32 °C			59.7	58.9	59.5	55.9	58.2
SEM			1.1	1.1	0.8	1.1	0.8
Statistical Probabilities							
Vitamin C			NS	NS	NS	NS	NS
Temperature			NS	NS	NS	NS	NS
Vitamin C x Temperature			NS	NS	NS	NS	NS

Table 4. Effect of dietary treatments on feed conversion ratio of layers exposed to heat stress

Diet	Vitamin C (g/l)	Temperature (°C)	W1	W2	W3 (g: g)	W4	Cumulative
1	0	22	1.889	1.769	1.822	1.801	1.811
2	0	32	1.977	1.918	1.898	2.099	1.998
3	0.2	22	1.776	1.669	1.728	1.835	1.782
4	0.2	32	1.922	1.905	1.877	1.938	1.907
SEM			0.062	0.068	0.054	0.069	0.052
Vitamin C average							
without			1.933	1.843	1.860	1.950	1.905
With			1.849	1.787	1.803	1.887	1.845
SEM			1.1	1.1	0.8	1.1	0.8
Temperature average							
22 °C			1.832	1.719 ^b	1.776 ^b	1.818 ^b	1.797 ^b
32 °C			1.950	1.911 ^a	1.888 ^a	2.019 ^a	1.953 ^a
SEM			0.044	0.048	0.038	0.049	0.037
Statistical Probabilities							
Vitamin C			NS	NS	NS	NS	NS
Temperature			NS	0.008	0.046	0.007	0.005
Vitamin C x Temperature			NS	NS	NS	NS	NS

Table 5. Effect of dietary treatments on egg shell strength of layers exposed to heat stress

Diet	Vitamin C (g/l)	Temperature (°C)	W1	W2	W3 (mm)	W4	Cumulative
1	0	22	4.28	4.19	4.65	4.44	4.39
2	0	32	3.92	3.49	3.71	4.13	3.81
3	0.2	22	4.42	4.31	4.21	4.72	4.42
4	0.2	32	4.24	3.98	4.26	4.20	4.17
SEM			0.30	0.15	0.17	0.17	0.10
Vitamin C average							
without			4.10	3.84	4.18	4.28	4.10
with			4.33	4.15	4.24	4.46	4.29
SEM			0.21	0.11	0.12	0.12	0.07
Temperature average							
22 °C			4.35	4.25	4.43	4.58	4.40
32 °C			4.08	3.75	3.99	4.16	3.99
SEM			0.21	0.11	0.12	0.12	0.07
Statistical Probabilities							
Vitamin C			NS	NS	NS	NS	NS
Temperature			NS	0.002	NS	0.02	0.0004
Vitamin C x Temperature			NS	NS	0.0009	NS	NS

Table 6. Effect of dietary treatments on blood mineral content of layers exposed to heat stress.

	Treatment				SEM	Vit C		SEM	Temperature		SEM	Probability		
	T1	T2	T3	T4		0	0.2		22°C	32°C		Vit. C	Tem.	Vit C x Tem.
Mineral concentration														
Na (mmol/l)	6.6	2.0	13.6	18.5	4.6	4.3	16.1	3.29	10.1	10.2	3.2	0.03	NS	NS
Cl (mmol/l)	99.3	99.0	96.3	94.5	3.2	99.1	95.4	2.29	97.8	96.7	2.3	NS	NS	NS
Mg (mmol/l)	3.4	3.2	3.4	2.9	0.17	3.3	3.1	0.12	3.43	3.08	0.12	NS	NS	NS
Ca (mg/ dl)	18.2	12.7	19.7	15.4	1.9	15.4	17.5	1.37	18.9	14.0	1.37	NS	0.034	NS
P (mg/ dl)	9.7	8.4	13.0	9.8	0.69	9.1	11.4	0.19	11.4	9.1	0.49	0.01	0.01	NS

Na: sodium, Cl: chloride, Mg: magnesium, Ca: calcium and P: phosphorus.

Table 7. Effect of dietary treatments on blood hematology of layers exposed to heat stress.

	Treatment				SEM	Vit C		SEM	Temperature		SEM	Probability		
	T1	T2	T3	T4		0	0.2		22°C	32°C		Vit. C	Tem.	Vit C x Tem.
Hematology														
WBC ($\times 10^3/\mu\text{L}$)	116.1	115.5	116.7	117.6	3.3	115.8	117.1	2.3	116.4	116.5	2.3	NS	NS	NS
RBC ($\times 10^6/\mu\text{L}$)	4.0	3.9	3.5	3.8	0.18	3.9	3.6	0.1	3.7	3.8	0.13	NS	NS	NS
HGB (%)	35.9	34.7	33.4	36.8	1.5	35.3	35.1	1.0	34.6	35.7	1.0	NS	NS	NS
MCV (fL)	111.6	111.6	112.9	113.3	1.9	111.6	113.1	1.3	112.2	112.4	1.38	NS	NS	NS
MCH (pg)	88.9	88.1	94.3	96.2	1.1	88.5	95.2	0.84	91.6	92.1	0.84	0.0005	NS	NS
MCHC (gHb/100 ml)	79.6	79.0	83.5	84.4	1.0	79.3	83.9	0.73	81.6	81.7	0.73	0.002	NS	NS
RDW-CV (%)	9.3	9.6	9.5	9.7	0.2	9.5	9.6	0.17	9.4	9.6	0.17	NS	NS	NS
RDW-CD (fL)	41.6	43.0	43.3	44	1.3	42.3	43.6	0.97	42.5	43.5	0.97	NS	NS	NS
HCT (%)	45.1	44.0	39.9	43.4	2.3	44.5	41.7	1.6	42.5	43.7	1.65	NS	NS	NS
PLT ($\times 10^3/\mu\text{L}$)	87.6	101.6	111.6	114.0	22.6	94.6	112.8	16.0	99.6	107.8	16	NS	NS	NS
MPV (fL)	10.8	11.1	11.3	10.3	0.2	10.9	10.8	0.14	11	10.7	0.14	NS	NS	0.01
PDW (%)	13.8	13.2	14.3	12.5	0.8	13.5	13.4	0.58	14	12.8	0.58	NS	NS	NS
PCT(%)	0.09	0.11	0.12	0.12	0.02	0.10	0.12	0.02	0.11	0.11	0.02	NS	NS	NS

WBC: white blood cell counts, RBC: total red blood cell counts, HGB: hemoglobin content, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW-SD: standard deviation in red cell distribution width, RDW-CV: coefficient variation of red cell distribution width, HCT: hematocrit, PLT: platelet count, MPV: mean platelet volume, PDW: platelet distribution width, PCT: plateletcrit

Conclusion: High environmental temperature reduced feed intake and increased FCR for the cumulative period of the experiment. In the contrary, vitamin C supplementation had no influence on feed intake or FCR. Egg shell strength was deteriorated with high temperature. Vitamin C increased Na and P concentration in the plasma and decreased Ca and P concentrations in hens subjected to HS.

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