Effects of Dietary Vitamins C and E on Egg Shell Quality of Broiler Breeder Hens Exposed to Heat Stress

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ABSTRACT : A feeding trial was conducted to determine whether dietary vitamin C (200 mg/kg) and vitamin E (250 mg/kg) prevent any drops in egg shell quality under heat stress in broiler breeder hens. One hundred and sixty molted Ross broiler breeders were housed randomly in an individual cage at 83 weeks of age. Four dietary treatments with forty hens and four replications per treatment were control (no additional vitamins), vitamin C-, or vitamin E-supplemented and combined supplementation of the two vitamins. After a ten-day-adaptation period at 25°C, the ambient temperature was kept at 32°C for a three-week-testing period. Egg production dropped dramatically over week but it did not show a significant change among treatments ($p<0.05$). However, egg quality parameters such as egg weight, specific gravity, shell thickness, SWUSA, puncture force and shell breaking strength from the birds fed the diet with the combined vitamins C and E were significantly improved over those of the control group during the heat stress period ($p<0.05$). The hens fed the vitamin C diet improved tibia breaking strength (37.16 kg), statistically higher than the birds fed the control and the vitamin E diets ($p<0.05$). The hens fed the control diet showed higher serum corticosterone levels, a mean of 5.97 ng/ml, than those of the other treatments ($p<0.05$). The heat stress resulted in elevated heterophils and decreased lymphocytes in serum, increasing the H/L ratios for all the treatments. However, the increases in H/L ratios were alleviated by feeding the diets containing vitamin C alone or together with vitamin E, although there were no significant differences in the ratio between the two groups ($p<0.05$). In conclusion, vitamins C (200 mg/kg) and/or E (250 mg/kg) supplemented to the diets for broiler breeder hens could prevent drops in egg shell quality and tibia bone strength under highly stressful environmental temperatures. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 4 : 545-551)***

Key Words : Vitamin C, Vitamin E, Egg Shell Quality, Heat Stress, H/L Ratios, Broiler Breeder Hen

INTRODUCTION

Stressful conditions from high environmental temperature have been a major concern to broiler and egg producers, particularly in summer. One of the adverse effects associated with high temperature is a reduction in the productive performance of laying hens with a high incidence of mortality (Thaxton and Pardue, 1984; Pardue et al., 1985; Mururi and Harrison, 1991). The decreased performances include lowered egg production, fertility and hatchability as well as increased incidence of thin shell eggs and sub-optimal albumin quality (Bains and Brake, 1995).

Environmental stress can lead to a reduction in the bird's defense mechanisms or suppressed immune response. In stress, many of the same hormones, glucocorticoids and epinephrine, are produced as in infection (Nockels, 1991). Many stresses can lead to production of oxygen free radicals in the body and the reactive radicals may damage cells by lipid peroxidation of polyunsaturated fatty acids. In a normal bird, there is sufficient antioxidant capacity to remove active oxygen but when exposed to environmental stress this may be depressed. Vitamin E, through its intra-membrane antioxidant properties, may protect tissue membranes from lipid peroxidation caused by free radical attack and thus alleviate the effects of environmental stress in laying hens (Smith, 1999). A series of studies showed consistent benefits from feeding additional vitamin E to laying hens experiencing heat stress. Dose response works suggested that 250 mg vitamin E is optimal for alleviating at least, in part, the adverse effects of chronic heat stress in laying hens (Bollengier-Lee et al., 1999).

Whitehead et al. (1990) showed the depression in egg production and feed intake which occurred at the higher temperature 35°C was overcome when hens were fed a diet containing 500 ppm of vitamin C. Vitamin C supplementation was beneficial to maintain hens performances including interior and exterior egg quality under severe environmental stresses (Cheng et al., 1990; Zapata and Gernat, 1995). The decline in egg shell quality is not only affected by the decreased intake of calcium and phosphorus but also by the depletion of ascorbic acid required for the conversion of 25-hydroxyvitamin D_3 produced by the liver into the hormone calcitriol produced in kidney, which is essential for regulation of calcium metabolism and egg shell calcification (Dorr and Balloun, 1976; Brake, 1988). Ahmad et al. (1967) concluded that vitamin C was helpful in maintaining egg quality characteristics, body temperature and metabolic activity in laying hens under high temperature conditions.

Studies on humans and animals have also shown that vitamin C is involved in bone formation by enhancing the production of hydroxyproline, which is necessary for

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Table 1. Composition and calculated nutrients contents of the basal diet

Ingredients	Percent
Corn	53.99
Wheat	10.00
Soybean meal	23.24
Rice bran	2.00
Animal fat	0.70
Tricalcium phosphate	1.16
Limestone	8.04
NaCl	0.30
Vitamin premix[1]	0.12
Mineral premix[2]	0.12
Choline chloride (25%)	0.20
DL-methionine (99%)	0.03
Vermicide[3]	0.04
Phytase[4]	0.06
Calculated nutrients	
AME[5] (kcal/kg)	2,760
Crude protein (%)	16.00
Calcium (%)	3.25
Available phosphorus (%)	0.43
Lysine (%)	0.82
Total S-containing amino acids (%)	0.58

[1] Vitamin premix provided per kilogram of diet: vitamin A acetate, 15,600 IU; vitamin D3, 120 ICU; dl-alpha-tocopherol acetate, 50 IU; menadione sodium bisulfite, 3.6 mg; vitamin B2, 6 mg; vitamin B_{12}, 0.024 mg; niacin, 48 mg; d-calcium panthothenate, 12 mg; folic acid, 1.8 mg; d-biotin 0.25 mg; ethoxyquin, 0.5 mg.

[2] Mineral premix provided per kilogram of diet: iron, 96 mg; manganese, 36 mg; zinc, 24 mg; copper, 12 mg; cobalt, 0.42 mg; iodine 0.72 mg; selenium, 0.12 mg.

[3] Larvadex™ (Novatis, Basel, Switzerland); as cyclomarzine 3 mg/kg diet.

[4] Natuphos™, 5,000 G/IU (BASF, Ludwigshafen, Germany).

[5] Apparent metabolizable energy.

collagen synthesis. The collagen fibril network is required for proper bone and egg shell formation (Weiser et al., 1988). During heat stress, however, vitamin C supply from the endogenous biosynthetic sources can be inadequate to meet all physiological requirements. Therefore, vitamin C available for reproductive function is limited, resulting in formation of thin shelled eggs (Bains and Brake, 1995).

Corticosteroid concentration in the blood has been used as a measure of environmental stress and physiological activity in chickens (Edens and Siegel, 1975; Siegel, 1980). Gross and Siegel (1983) found that the number of lymphocytes in chicken blood samples decreased and the number of heterophils increased in response to stressors and to increasing levels of corticosterone in the chicken feed. The heterophil/lymphocyte (H/L) ratio appears to be a more reliable indicator of levels of corticosterone in the feed and to social stress than were the plasma corticosterone levels.

This study was conducted to investigate the effects of dietary vitamins C (200 mg/kg) and E (250 mg/kg) on egg shell quality and bone parameters of broiler breeder hens exposed to constant high environmental temperature. We also wanted to find out any synergistic effects of the two vitamins supplemented together on alleviating the decreased overall performances relating to egg shell and bone development of the hens.

MATERIALS AND METHODS

One hundred sixty molted Ross broiler breeder hens, 83 wk of age and with a 60% average egg production rate, were randomly assigned in individual cages. The cage dimensions were 30 cm wide×45 cm deep. After a ten-day-adaptation period at room temperature (about 25°C), the ambient temperature of the house was raised to 32°C with automatic electrical heaters and kept constant within a range of ±3°C during a three-wk experimental period. The hens were exposed to 55-60% RH set by commercial humidifiers and a photoperiod of 16L:8D throughout the period. The birds were manually fed the diets, and water was freely available during the whole period. Eggs were collected and weighed individually everyday. A commercial broiler breeder diet formulated basically to satisfy NRC (1994) recommendations was provided to all birds during the adaptation period on a restricted feeding program suggested by the breeder management guide. Dietary formulation and calculated nutrients compositions of the diet were as shown in Table 1.

Birds were allotted into four dietary groups with four replications and ten birds each. The four dietary groups were control (basal diet without additional vitamins), diets supplemented with vitamin C (200 mg/kg) or vitamin E (250 mg/kg), and a diet supplemented with the two vitamins at each level (vitamin C+E). The basal diet was a commercial broiler breeder hen diet already containing 50 IU vitamin E per kg diet from the vitamin premix.

Egg shell quality measurements

All eggs were collected daily and egg shell quality parameters such as egg weight, specific gravity, egg shell puncture force, SWUSA (shell weight per unit surface area), breaking strength, and thickness were measured on a weekly basis. Feed intake, egg production and mortality were recorded weekly.

Egg specific gravity : Specific gravity of the whole egg was measured according to Archimedes' principle (Watkins and Southern, 1992).

Shell weight per unit surface area (SWUSA) : SWUSA was calculated by dividing the shell weight with adhering membranes (mg) by the egg surface area (cm^2) as described by Ousterhout (1980).

Shell thickness : Shell thickness at the equatorial region was measured with triplicate to 0.001 mm accuracy with a dial thickness gauge (Mitutoyo Corporation, Japan). Shell thickness was measured without the membrane (outer) after

drying. Membranes were removed by boiling the egg shells in 5% NaOH solution for 10 min.

Compressive breaking strength : Shell breaking strength was determined using an Instron (Model 4465. Instron Corp., Canton, MA) according to Voisey and MacDonald (1978). The apparatus with 50 kg load cell was arranged to operate in the vertical position for either compression or puncture testing in "top-down" position. Tests were conducted by compression surfaces, a stationary surface and a moving cylindrical stainless steel punch, at a constant crosshead speed of 2.0 mm/min.

Puncture force : Dried egg shell pieces (1×1 cm) were prepared using a diamond-blade cutter for puncture force measurement. The measuring procedure is the same as breaking strength except using a 5 kg load cell and a 1 mm probe.

Blood components and bone measurements

Blood samples to measure white blood cell (WBC) counts were taken from wing veins between 1100 h and 1300 h from six birds each treatment every week. Cautions were applied to minimize physiological variations by taking blood only from hens carrying a shelled egg in the uterus, determined by palpation. At the end of the study, four hens from each treatment were sacrificed by cervical dislocation between 1100 h and 1300 h. Blood serum from the birds were used to determine vitamin C, alpha-tocopherol and corticosterone. Tibias were sampled as described by Orban (1993) to measure bone parameters such as tibia weight, length, and breaking strength.

H/L ratios : The numbers of heterophils and lymphocytes were measured from a blood smear prepared by centrifugation with a Larc Spinner (Corning Glass Works, Scientific Instrument Division, Metfield, MA). Smears were stained within seven days of preparation with May-Grunwald-Giemsa stain. The total leukocyte count included heterophils, lymphocytes, monocytes, basophils, and eosinophils. About 100 cells were counted for each data. Heterophil/lymphocyte (H/L) ratios were determined by dividing the number of heterophils by the number of lymphocytes (Gross and Siegel, 1983).

Serum concentrations of vitamin C and vitamin E : Vitamin C concentration was determined according to the method described by Harapanhalli et al. (1993). After deproteinizing samples with 5 mM metaphosphoric acid containing 5 mM EDTA, supernatant was injected onto a C18 column. Vitamin C was separated by a reverse-phase HPLC using an isocratic gradient of 0.1 M NaH_2HPO_4 containing 0.2 mM Na_2EDTA (pH 3.1) and detected at 254 nm. The HPLC system consists of a Spectra-Physics Model 8100 gradient pump with a C18 Nova-Pak column (3.9×150 mm; Waters Corp., Milford, MA), and a UV/Vis detector (Model 8440, Spectra-Physics).

Vitamin E analysis was accomplished using a modified method of Bottje et al. (1997). Briefly, protein was precipitated in duplicate aliquots using ice-cold ethanol containing ascorbic acid (1 g/L). After extracting the serum sample twice with 2 ml hexane, the combined organic layer was evaporated under nitrogen. Vitamin E was re-dissolved in methanol/acetonitrile (1:3), centrifuged for 5 min at 12,000×g and 20 µl of the supernatant used for liquid chromatography. For vitamin E analysis, the same reverse-phase HPLC system with a fluorescence detector (Waters Corp., Milford, MA) set at 298 and 328 nm for emission and excitation wavelengths, respectively, was used.

Corticosterone concentration in serum : Corticosterone (C-2505, Sigma Chemicals Co., St. Louis) in methanol/water (60:40) solution was used to prepare standard curves. Samples were extracted and prepared according to Fowler et al. (1983). The absorbance of corticosterone in the elute was determined with a UV detector (Model 8840, Spectra-Physics) at 248 nm.

Tibia bone breaking strength : The tibias were individually sealed in a plastic bag to minimize moisture loss, and stored at -20°C until analyzed. Wet bones stored at -20°C have no effects on bone strength determinations (Seldin, 1965). The weight and length were measured with fresh bones. To measure bone breaking force with an Instron (Model 4465, Automated Materials Tester), each bone was supported by a fulcrum with 9 cm-width. A probe of 6 cm length and a round base were attached to a 500 kg load cell with a crosshead speed of 200 mm/min.

Statistical analysis

Statistical comparison among the experimental groups was performed by one-way ANOVA using the General Linear Models procedure of the Statistical Analysis System (SAS Institute, 1996). When ANOVA was significant, the Tukey's test was applied for the *post hoc* comparisons. All statement of significance was based on the probability of no difference between means being less than p=0.05.

RESULTS AND DISCUSSION

Overall production performances including livability of the breeder hens decreased remarkably by the heat exposure (Table 2). High mortality (10-20%) of the birds during the first week of the stress was the most significant in all dietary groups. In numerical terms, control group fed the basal diet showed the highest mortality rate (20%), followed by, in the order of, vitamin C (15%), vitamin E (12.5%) and vitamin C+E groups (10%), with no statistically significant difference. However, none of the groups showed any mortality from the second week of the heat stress. Egg production rate of the birds decreased drastically depending on the length of time exposed to the

Table 2. Weekly performances of broiler breeder hens fed the experimental diets with various levels of vitamins C and E and exposed to high environmental temperatures of 32°C for 3 weeks

Treatments	Egg weight (g)	Egg production[2] (%)	Feed intake (g/bird/day)	Mortality (%)
Week 1				
Basal diet (B.D)	62.6±1.9	41.3±6.0	129.2±5.0	20.0±14.4
B.D.+vitamin C[3]	62.8±1.4	51.3±16.8	131.9±8.3	15.0±12.9
B.D.+vitamin E[4]	63.5±1.9	45.4±8.8	131.2±16.6	12.5±9.6
B.D.+C+E[5]	64.9±2.0	52.1±3.7	127.5±11.0	10.0±8.2
Week 2				
Basal diet (B.D)	62.7±1.7	20.3±10.0	128.4±8.7	0
B.D.+vitamin C[3]	62.6±2.2	24.4±14.0	136.3±23.9	0
B.D.+vitamin E[4]	62.5±0.8	25.3±9.6	137.7±11.9	0
B.D.+C+E[5]	64.5±3.6	34.9±7.3	137.7±3.6	0
Week 3				
Basal diet (B.D)	60.3±1.8	11.7±9.7	126.7±10.3	0
B.D.+vitamin C[3]	62.6±3.8	17.1±12.5	126.2±10.6	0
B.D.+vitamin E[4]	61.1±2.9	17.7±12.3	134.0±23.7	0
B.D.+C+E[5]	66.2±5.7	28.3±5.4	125.8±8.2	0

[1] Values are mean values±standard deviation. [2] Egg production: hen-day egg production.
[3] Basal diet−vitamin C (200 mg/kg diet). [4] Basal diet+vitamin E (250 mg/ kg diet).
[5] Basal diet−vitamin C (200 mg/kg)+vitamin E (250 mg/kg).

Table 3. Changes of various egg quality parameters from broiler breeder hens before and after exposed to high environmental temperatures at 32°C for 3 weeks[1]

Treatment	Egg weight (g)	Specific gravity	Breaking strength (kg)	Puncture force (kg)	SWUSA[2] (mg/cm^2)	Thickness (mm)
Before[3]						
Basal diet	63.5±4.7	1.066±0.005	3.19±0.53	0.356±0.042	74.3±4.7	0.352±0.015
B.D.+vit.C[5]	64.8±4.6	1.062±0.008	3.02±0.48	0.354±0.047	70.7±8.1	0.335±0.034
B.D.+vit.E[6]	63.7±4.7	1.066±0.005	2.98±0.33	0.359±0.040	72.1±6.9	0.343±0.035
B.D.+C+E[7]	65.3±3.8	1.063±0.011	3.05±0.64	0.368±0.054	71.6±11.1	0.343±0.047
After[4]						
Basal diet	62.2±4.9^{a}	1.062±0.010^{a}	2.62±0.64^{a}	0.338±0.041^{a}	68.6±7.7^{a}	0.327±0.034^{a}
B.D.+vit.C[5]	63.6±4.2^{b}	1.065±0.009^{ab}	2.86±0.43^{ab}	0.365±0.038^{ab}	70.3±5.2^{ab}	0.333±0.028^{ab}
B.D.+vit.E[6]	62.6±4.9^{ab}	1.065±0.011^{ab}	2.81±0.49^{ab}	0.360±0.039^{ab}	70.7±8.9^{ab}	0.334±0.040^{ab}
B.D.+C+E[7]	65.5±3.9^{b}	1.068±0.009^{b}	3.07±0.49^{b}	0.380±0.045^{b}	72.6±7.1^{b}	0.346±0.033^{b}

[a,b] Means in a column with no common superscripts are significantly different ($p<0.05$).
[1] Values are means±standard deviation of thirty samples. [2] SWUSA: shell weight per unit surface area.
[3] One week prior to exposure to high temperature. [4] Mean values during the 3 wk exposed to high temperature.
[5] Basal diet−vitamin C (200 mg/kg). [6] Basal diet−vitamin E (250 mg/kg).
[7] Basal diet−vitamin C (200 mg/kg)+vitamin E (250 mg/kg).

heat stress. Weekly means of the egg production rate decreased consecutively from 47.5 for the first week to 26.2 and 18.7% for the second and third weeks, respectively. The birds of the vitamins C+E group tended to maintain better egg production rate during the second and third week compared to the others, and the production rate of the vitamins C+E group in the third week was significantly better than that of the control group ($p<0.05$).

Egg weight and feed intake of all dietary groups were not affected by the heat exposure, indicating that heat stress decreased total egg mass but not egg weight produced by the breeder hens. This is in contrast to Mururi and Harrison (1991) who reported, in laying hens, larger differences in egg weight during thermo-neutral zone and heat stress periods. The discrepancy between the latter and present studies could be related to the differences in feed intake. No changes in feed intake in the broiler breeder hens on restricted feeding program and the lowered egg production rate in this study could be the reasons to support normal egg weight. The observation that no mortality was found from the second week of the heat stress suggests an adaptation of the birds to the stress by then (Table 2).

Data in Table 3 showed that heat stress tended to deteriorate all kinds of the egg shell quality parameters including egg weight, specific gravity, breaking strength, puncture force, SWUSA, and shell thickness. For one week prior to heat exposure, the egg qualities were not affected at all by dietary treatments. General responses in the Table 3 indicated that the vitamin C+E diet tended to maintain the egg quality better than the other dietary groups when exposed to the heat stress. For all six parameters in the Table 3, the vitamins C+E group showed significantly better

Table 4. Effects of dietary vitamins C and E on tibia bone measurements from broiler breeder hens exposed to high environmental heat stress at 32°C over three weeks[1]

Treatment	Body weight[2] (g)	Tibia		
		Weight (g)	Length (cm)	Breaking strength (kg)
Basal diet	3,762±343	22.81±0.77	12.2±0.4	29.91±2.12[a]
B.D.+vitamin C[3]	3,890±254	23.44±0.87	12.2±0.2	37.16±2.91[b]
B.D.+vitamin E[4]	3,889±391	22.52±1.03	12.2±0.2	29.22±7.86[a]
B.D.+C+E[5]	4,049±451	23.59±0.67	12.3±0.1	33.52±4.75[ab]

[a, b] Means in a column with no common superscripts are significantly different ($p<0.05$).
[1] Values are mean values = standard deviation of eight samples.
[2] Mean body weight of the birds sampled. [3] Basal diet−vitamin C (200 mg/kg diet).
[4] Basal diet−vitamin E (250 mg/kg diet). [5] Basal diet+vitamin C (200 mg/kg diet)−vitamin E (250 mg/kg diet).

Table 5. Weekly changes of differential white blood cell concentrations in broiler breeder hens exposed to high environmental temperatures at 32°C[1]

Treatment	Week 1 (%)	Week 2 (%)	Week 3 (%)
Heterophil			
Basal diet	64.5±5.7	59.3±4.8	58.0±5.9
B.D.+C[2]	57.8±5.9	54.3±6.5	53.8±5.6
B.D.+E[3]	56.8±3.3	60.3±5.7	55.8±7.2
B.D.+C+E[4]	52.8±6.9	54.8±6.6	54.8±4.8
Lymphocyte			
Basal diet	29.5±5.5	29.3±7.5	31.0±4.7
B.D.+C[2]	35.6±5.4	35.5±8.2	36.0±4.5
B.D.+E[3]	36.4±4.1	31.0±8.9	31.4±7.0
B.D.+C+E[4]	39.4±8.8	37.0±5.3	34.8±2.4
H/L ratio			
Basal diet	2.3±0.6	2.5±1.2	1.9±0.4
B.D.+C[2]	1.7±0.4	2.6±0.2	1.5±0.3
B.D.+E[3]	1.6±0.3	2.5±0.4	1.9±0.9
B.D.+C+E[4]	1.5±0.5	1.5±0.4	1.6±0.2

[1] Values are mean values±standard deviation (n=5). No significant differences exist among the dietary groups and weekly values. Heterophils, lymphocytes and H/L ratio before the heat exposure were 33.7, 59.7 and 0.56, on the averages, respectively.
[2] Basal diet−vitamin C (200 mg/kg diet).
[3] Basal diet−vitamin E (250 mg/kg diet).
[4] Basal diet−vitamin C (200 mg/kg diet)+vitamin E (250 mg/kg diet).

results than those of the control group ($p<0.05$). Breaking strength of the eggs from the control group dropped significantly from 3.19 to 2.63 kg by the stress. However, the strength values of the vitamins C+E group were not decreased even after the heat exposure, compared to those of the other groups ($p<0.05$). Similar observations were found for the other measurements.

We tried to obtain various measurements of egg shell quality because no one parameter provides the most representative results in changes of egg shell quality (Table 3). The simplest method of evaluating egg shells is to measure their thickness, either directly and indirectly by means of specific gravity. Direct measurements require broken shells and are subject to error because of variation in thickness at different points on the shell. Measuring the specific gravity can avoid breaking the egg, but is subject to errors such as temperature and calibration of the equipment. Breaking strength measurement requires specific equipment such as Instron and bears little relation to the type of stress likely to encounter in the field. Shell weight per unit surface area (SWUSA) is highly recommended by Sauveur and Picard (1987) as a factor proportionally well affected by the environmental heat stress. Puncture force is to simulate a condition in the field when the egg is in contact with a sharp edge of the equipments (Hunton, 1987). Overall responses data in Table 3 are showing relatively good agreement between various measurements for the eggs from the hens exposed to the heat stress.

The decreases in egg shell quality such as shell thickness and specific gravity are normal responses in hens exposed to heat stresses (Muiruri and Harrison, 1991). Many studies reported that supplementation of vitamin C to the hen diets could alleviate the decreases in egg production rate and egg quality (Bendich et al., 1984; Cheng et al., 1991).

Tibial bone breaking strength in Table 4 appeared the highest for the hens fed the vitamin C diet with significant differences compared to those for control and vitamin E groups ($p<0.05$). The bone strength value for the vitamin C was 37.16 kg, followed by 33.52 kg for the vitamins C+E group. 29.91 and 29.2 kg for control and vitamin E groups, respectively. Tibial bone weight and length were not affected by dietary treatments. The higher bone breaking strengths of the birds fed the diets added with either vitamin C alone or together with vitamin E likely support that the vitamin is an essential factor for collagen formation and contributes to renal activation of vitamin D_3 for bone development (Borr and Balloun, 1976; Weiser et al., 1988). Lohakare et al. (2004a) observed that tibia bone resistance was significantly higher in vitamin C supplemented groups than the control group in broiler chickens. They (2000b) also found that vitamin E supplementation improved tibia bone resistance statistically in broilers. Higher calcium and phosphorus content in bone was noted in the higher vitamin E-fed group.

Table 5 showed weekly changes of the white blood cell levels of the hens exposed to the heat stress. In general, there were no significant differences in the levels of heterophils, lymphocytes and H/L ratios among the dietary groups each week and among the weekly intervals. Just in numerical terms, the vitamins C+E groups tended to show

Table 6. Serum concentration of vitamin C, vitamin E and corticosterone in broiler breeder hens exposed to high environmental heat stress at 32°C[1]

Components	Basal diet	BD+vit.C[2]	BD+vit.E[3]	BD+C+E[4]
Vitamin C (g/ml)	7.87±1.82[a]	12.73±1.80[b]	7.26±0.92[a]	12.24±1.22[b]
Vitamin E (g/ml)	2.80±0.14[b]	1.63±0.26[a]	8.20±0.21[c]	8.35±0.13[c]
Corticosterone (ng/ml)	5.97±1.42[a]	3.23±0.70[b]	2.54±0.23[b]	2.78±0.61[b]

[a-c] Means in a row with no common superscripts are significantly different ($p<0.05$).

[1] Values are mean values±standard deviation of three samples. [2] Basal diet+vitamin C (200 mg/kg diet).

[3] Basal diet+vitamin E (250 mg/ kg diet). [4] Basal diet+vitamin C (200 mg/kg)+vitamin E (250 mg/kg).

the lower H/L ratios (1.5-1.6) compared to the control (1.9-2.5). The H/L ratio value obtained before the heat exposure was 0.56 and numbers of heterophils and lymphocytes during the same period were 33.7 and 59.7%, respectively. The observation that the blood profiles of the birds one week after exposed to the heat stress did not show anymore changes might reflect the adaptation of the bird blood cells within a week.

Blood levels of the vitamins C and E, together with corticosterone are as show in Table 6. As expected serum levels of the two vitamins were significantly elevated when the birds were fed the diets supplemented with such vitamins ($p<0.05$). Corticosterone level in the control group appeared higher than those in the other groups with a significance at $p<0.05$, suggesting that the release of stress hormone could be under control by feeding the diets supplemented with higher levels of the vitamins. Corticosteroid concentration in the blood and H/L ratios have been used as measures of environmental stress and physiological activity in chickens. The H/L ratio was suggested as a more reliable indicator to social stress than the plasma corticosterone levels. (Edens and Siegel, 1975; Siegel, 1980; Gross and Siegel, 1983). However, the observation in this study suggested that H/L ratio could be a good indicator for short period of time (within week) and corticosterone levels could be useful indicator for long term purposes. Important observation in this study is that the diet supplemented with vitamins C+E tended to show better egg quality under the severe heat stress compared to the diets supplemented with single vitamin C or E.

We can suggest a couple of speculations for their roles and relationships between the vitamins C and E from this study. Firstly, the level of vitamin C added to the diet might not enough to play a role in preventing the egg quality decrease under the conditions of present study. Whitehead et al. (1990) once reported that 500 ppm vitamin C was required to overcome the depression in egg production when hens were heat exposed. Secondly, vitamin E acted as another antioxidant to spare the vitamin C so that the effective levels of vitamin C could be maintained in the tissues, implying their synergic effect as suggested by Sahin et al. (2002) in laying hens at high ambient temperature. Thirdly, vitamin C could have sparing action for vitamin E. Vitamin C can react with the free radical of vitamin E to regenerate the reduced form of the vitamin E, meanwhile the vitamin C molecule being oxidized (Niki et al., 1982; Bendich et al., 1984). The net result is a decrease in tissue levels of vitamin C and maintenance of the antioxidant vitamin E levels. The serum levels of the two vitamins in Table 6 hardly show any support for this speculation. Final speculation is that vitamin E could have its own unique function in keeping the normal physiology under such a stressful condition. Vitamin E seemed to play an important role in cellular metabolism under heat stress. Bollengier-Lee et al. (1998, 1999) indicated metabolic differences due to a high level of vitamin E supplementation during a heat stress. Further studies are required to clarify the relationships between the vitamins C and E, and along with other antioxidative substances in their effectiveness to alleviate deteriorating egg quality associated with heat stress.

In conclusion, vitamins C (200 mg/kg) and/or E (250 mg/kg) supplementation to diets could prevent decreases in egg shell quality and tibia bone strength by alleviating stressful effects from high environmental temperature in broiler breeder hens.

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