Effects of Dietary Vitamins C and E on Egg Shell Quality of Broiler Breeder Hens under 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 ℃, the ambient temperature was kept at 32 ℃ 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 weight, SG, shell thickness, SWUSA, puncture force and shell breaking strength of the birds fed the diet with the combined vitamins C and E were significantly improved than those fed the basal diet during the heat stress period ($P<0.05$).

The hens fed the vitamin C supplemented diet showed a tibia breaking strength of 37.16 kg statistically higher than those of the basal and the vitamin E supplemented groups ($P<0.05$). The hens fed the basal diet showed higher serum corticosterone levels, a mean of 5.97 ng/ml, than those of the rest of treatments ($P<0.05$). The heat stress elevated heterophils but decreased lymphocytes in serum, and it changed H/L ratios of all the treatments. The increases in H/L ratios were alleviated in the bird by feeding vitamin C and/or vitamin E supplemented diets, but they did not differ significantly ($P<0.05$). In conclusion, vitamins C (200 mg/kg) and/or E (250 mg/kg) supplementation to diets could prevent drops in egg shell quality and tibia bone strength by alleviating stressful effects from high temperature in broiler breeder hens.

Key words : Vitamin C, Vitamin E, Egg shell quality, Broiler breeder hens, Heat stress, H/L ratios

Introduction

High environmental temperature is major concern to broiler and egg producers. The literature indicates a number of adverse effects associated with high environmental temperature. A reduction in the productive performance of laying hens with a high incidence of mortality has been reported by several authors (Thaxton and Pardue, 1984; Pardue *et al.*, 1985; Muruni and Harrison, 1991). In commercial broiler breeders the adverse effects of heat stress are limited not only to

increased mortality but also include decreased 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 birds 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. Reactive free 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 ℃ was overcome when hens were fed a diet containing 500 ppm of vitamin C. Cheng *et al.* (1990) suggested that vitamin C supplementation is beneficial to laying hen mortality caused by environmental stresses and improvements in both interior and exterior egg quality can also be realized with its use. 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 the regulation of calcium metabolism and egg shell calcification (Brake, 1988). During heat stress, however, ascorbic acid from the endogenous biosynthetic source is inadequate to meet all physiological requirements. Therefore, ascorbic acid available for reproductive function is limited which results in production of thin shell eggs (Bains and Brake, 1995).

Studies on humans and other animals have shown that vitamin C is involved in bone formation by enhancing the production of hydroxyproline, which is necessary for collagen formation. The collagen fibril network, which is required for proper bone and egg shell formation, has to be mineralized with hydroxyapatite. Some poultry studies have indicated that vitamin C may be stimulatory or synergistic with 1,25-dihydroxycholecalciferol production (Weiser *et al.*, 1988) and also may be involved in bone mobilization of calcium (Thornton, 1970; Dorr and Balloun, 1976).

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 effects

of dietary vitamin C (200 mg/kg) on egg shell quality and bone parameters of broiler breeder hens under heat stress and dietary vitamin E (250 mg/kg) on alleviating depression of egg shell quality by alleviating heat stress. And they were to determine if a combination of the two vitamins shows synergistic effects on egg shell quality.

Materials And Methods

(1) Birds and Management

One hundred and sixty molted Ross broiler breeders were housed randomly in an individual cage at 83 weeks of age. After a ten-day-adaptation period at 25 ℃, the ambient temperature of the house was kept continuously at 32 ℃ for a three-week-testing period.

The birds were provided a photoperiod of 16L:8D throughout the experiment period and the relative humidity ranged from 55 to 60 %. Water was made available for *ad libitum* consumption. Disturbance of birds was limited to routine husbandry procedures and experiment manipulations.

(2) Diets and Treatments

A commercial broiler breeder diet meeting or exceeding all National Research Council (1994) recommendations was fed throughout the experiment period. The calculated nutrients for the diet were provided in Table 1. The diet was provided on a restricted feeding program recommended by the breeders management guide. Four dietary treatments with forty hens and four replication per treatment were control (basal diet with no additional vitamins), vitamin C (200 mg/kg) supplemented, or vitamin E (250 mg/kg) supplemented and a combined supplementation of the two vitamins.

(3) Measurements

All the eggs laid were collected daily and egg shell quality parameters : egg weight, specific gravity by Archimedes method, egg shell puncture force, SWUSA, breaking strength, and thickness were measured on a weekly basis. Feed intake, egg production, egg weight, and mortality were also calculated weekly.

Table1. Composition and calculated nutrients content 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	
ME[5], kcal/kg	2,760.00
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 D_3 120 ICU,
dl-α -tocopherol acetate 50 IU, menadione sodium bisulfite 3.6 mg,
vitamin B_2, B_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™, 5000 G/IU (BASF, Ludwigschafen, Germany)

[5] ME = metabolizable energy

Shell weight per unit surface area (SWUSA) : SWUSA was calculated by dividing the shell weight adhering membranes (mg) by the egg surface area (cm^2) described by Ousterhout (1980). Egg surface area was calculated according to Carter (1975) using the equation : 3.9782 × egg weight (g). 7056.

Shell thickness : Shell thickness at the equatorial region was measured to 0.001 mm accuracy with a dial thickness gauge (Mitutoyo Co., Japan). Shell thickness was measured with and without the membrane after drying. Membranes were removed by boiling the egg shells in 5 % NaOH for 10 min. Membrane thickness was calculated by the difference in shell thickness with and without the membrane (Peebles and Brake, 1985).

Compressive breaking strength : Shell breaking strength was determined using an Instron Automated Materials Tester Model 4465 (Instron Co., Canton, MA 02021, USA) 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 applied 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. The measuring procedure is the same as breaking strength except using a 5-kg load cell and a 1-mm probe. Blood samples were taken from six birds each by treatment randomly selected for the determination of white blood cell (WBC) counts on the weekly basis after heating up to 32 ℃ from the ambient temperature at 25 ℃. Blood was drawn from wing veins between 1100 h and 1300 h and the differential WBC counts were measured by using a stained-slide method. Differences were minimized by taking blood only from hens that were known to contain a shelled egg in the uterus, as determined by palpation.

H/L ratios : The number of heterophils and lymphocytes was determined by examining a blood smear prepared by centrifugation with a Larc Spinner (Corning Glass Works, Scientific Instrument Division, Metfield, Masschusetts). Smears were stained within 7 days of preparation with May-Grunwald-Giemsa stain. The total leukocyte count includes heterophils, lymphocytes, monocytes, basophils and eosinophils. About 100 cells were counted for each ratio. In each cell-counting method, heterophil /lymphocyte (H/L) ratios were determined by dividing the number of heterophils by the number of lymphocytes. At the end of the experiment, a total of four hens each per treatment were killed by cervical dislocation between 1100 h and 1300 h. Blood was drawn from the killed birds and serum vitamin C, alpha-tocopherol and corticosterone were determined. Tibias were taken by the method described by Orban(1993) for measuring bone parameters: tibia weight, length, and breaking strength described below.

Serum concentrations of vitamin C, E : Vitamin C concentrations were 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 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 system with a C18 Nova-Pak column (3.9×150 mm; Waters Co., Milford, MA 01757, USA), and a Spectra-Physics Model 8440 UV / Vis detector. Vitamin E 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 ml of the supernatant used for liquid chromatography. A reverse-phase HPLC system using a Spectra-Physics Model 8100 gradient pump system with a C18 Nova-Pak column (3.9×150mm; Waters Co., Milford, MA 01757, USA), and a Waters fluorescence detector was used. And fluororescence detection was monitored using emission and excitation wavelengths of 298 and 328 nm, respectively.

Corticosterone concentration in serum : Corticosterone (C-2505, Sigma Chemicals Co., St. Louis, MO 63178) solutions in methanol/water 60:40 were prepared for the determination of standard curves. Samples were extracted according to Fowler *et al.*(1983). The concentration of corticosterone in the elute was determined by UV absorbance at 248 nm using a Spectra-Physics Model 8840 detector.

Tibia bone breaking strength : The tibias were individually sealed in a plastic bag to minimize moisture loss, and stored at -20 ℃ until analysis. Storage of wet bones at -20 ℃ has been reported to have no effect on various bone strength determinations (Seldin, 1965). The weighing accuracy was within 0.001 g and bone length was measured with a caliper with an accuracy of 0.001 cm. The tibias were thawed before breaking force was measured with Instron Automated Materials Tester Model 4465. Each tibia was supported by a fulcrum with 9 cm-width. A probe with a length of 6 cm and a round base was attached to 500-kg load cell with a crosshead speed of 200 mm/min.

(4) Statistical Analysis

Data were subjected to one-way analysis of variance. Statistical comparison among the groups in the experiment 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 Tukeys multiple-range test was applied for the *post hoc* comparisons. All statements of significance were based on the probability of no difference between means being less than $P=0.05$, unless otherwise noted.

Results And Discussion

Neither egg weight nor feed intake exhibited significant differences between all groups, whereas, egg production decreased remarkably in all groups. The rate of egg production in the vitamins C and E supplemented group dropped from 52.1 to 28.3 % but that of the basal diet group decreased from 41.3 to 11.7 % in two weeks. The highest mortality was found in the basal diet group at 20 % as compared with 10 % of the combined vitamins C and E supplemented group but no

mortality was observed for the next two weeks (Table 2). These results implicated that high temperature may cause a significant drop in egg production and high mortality rates after a sudden exposure to stressful high temperature. The results are similar to Whitehead *et al.*(1990) who showed the depression in egg production was overcome when hens were fed a diet containing 500 ppm of vitamin C and Cheng *et al.*(1990) who indicated that vitamin C supplementation is beneficial to laying hen mortality caused by environmental stresses.

Table 3 indicated that heat stress deteriorated all the egg shell quality parameters : egg weight, specific gravity, breaking strength, puncture force, SWUSA and thickness. The egg shell quality criteria of the combined vitamins C and E supplemented group were better than those of the

Table 2. Effects of dietary vitamins C and E on laying performance of broiler breeder hens under heat stress at 32 ℃ during a 3-week-testing period[1]

Treatment		Egg weight g	Egg production[2] %	Feed intake g/bird/day	Mortality %
Week 1	Basal Diet	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	15.9 ± 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	62.7 ± 1.7	41.3 ± 6.0	128.4 ± 8.7	0.0
	B.D.+vitamin C[3]	62.6 ± 2.2	51.3 ± 16.8	136.3 ± 23.9	0.0
	B.D.+vitamin E[4]	62.5 ± 0.8	45.4 ± 8.8	137.7 ± 11.9	0.0
	B.D.+C+E[5]	64.5 ± 3.6	52.1 ± 3.7	137.7 ± 3.6	0.0
Week 3	Basal Diet	60.3 ± 1.8	11.7 ± 9.7	126.7 ± 10.3	0.0
	B.D.+vitamin C[3]	62.6 ± 3.8	17.1 ± 12.5	126.2 ± 10.6	0.0
	B.D.+vitamin E[4]	61.1 ± 2.9	17.7 ± 12.3	134.0 ± 23.7	0.0
	B.D.+C+E[5]	66.2 ± 5.7	28.3 ± 5.4	125.8 ± 8.2	0.0

[1] Data are mean values± standard deviation of all eggs laid and feed intake of hens every day.
[2] Egg production: hen-day egg production
[3] Basal Diet + vitamin C (200 mg/kg)
[4] Basal Diet + vitamin E (250 mg/kg)
[5] Basal Diet + vitamin C (200 mg/kg) + vitamin E (250 mg/kg)

Table 3. Effects of dietary vitamins C and E on egg weight, specific gravity, breaking strength, puncture force, SWUSA, and thickness under heat stress at room temperature 32 ℃ for 3 weeks[1]

Treatment		Egg weight g	Specific gravity	Breaking strength kg	Puncture force kg	SWUSA[2] mg/cm^2	Thickness mm
Week 0	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[3]	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[4]	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[5]	65.3 ± 3.8	1.063 ± 0.011	3.05 ± 0.64	0.368 ± 0.054	71.6 ± 11.1	0.343 ± 0.047
Week 1 to 3	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[3]	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[4]	62.6 ± 4.9^{ab}	1.065 ± 0.011^a	2.81 ± 0.49^{ab}	0.360 ± 0.039^{ab}	70.7 ± 8.9^{ab}	0.334 ± 0.040^{ab}
	B.D.+C+E[5]	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] Data are mean values± standard deviation of thirty samples.
[2] SWUSA: shell weight per unit surface area
[3] Basal Diet + vitamin C (200 mg/kg)
[4] Basal Diet + vitamin E (250 mg/kg)
[5] Basal Diet + vitamin C (200 mg/kg) + vitamin E (250 mg/kg)

basal diet group during the testing period($P<0.05$). Breaking strength of the basal diet group dropped significantly to 2.629 from 3.19 kg in three weeks. However, breaking strength of the combined vitamins C and E supplemented group improved from 3.05 to 3.07 kg ($P<0.05$). The basal diet group showed a serious drop in shell thickness from 0.352 to 0.327 mm but the shell thickness of the vitamins C and E group improved to 0.346 from 0.343 mm, which differed statistically from that of the basal diet group ($P<0.05$). The values of egg weight, specific gravity, puncture force, and SWUSA were significantly higher in the vitamins C and E supplemented group than those in the basal diet group during the testing period($P<0.05$). There was no significant difference between the vitamin C and E supplemented group in all the criteria of egg shell quality. Payne(1966) found no difference in egg weight between 18 to 28 ℃ but there was a large reduction when environmental temperatures exceeded 32 ℃ and he indicated this could not be corrected by nutrient adjustments and it was attributed directly to heat stress. Also Muiruri and Harrison (1991) showed larger differences in egg weight during thermo-neutral and heat stress periods because both egg shell thickness and specific gravity were greatly reduced by heat stress conditions. The basal diet group in this study showed similar downward patterns in egg weight, shell thickness and specific gravity as compared with the above two reports. However, dietary vitamin C and / or E supplementation appeared to alleviate the deterioration of egg shell quality during the stressful high temperature at 32 ℃. Results are related to studies implicating that vitamin C is involved in collagen formation, which is required for proper bone and egg shell formation. In the present study it was difficult to suggest that vitamin E has a sparing effect of vitamin C on egg shell quality. However, the results in the study implied that vitamin E had a synergistic effect on egg shell quality with vitamin C. This implication is similar to Sahin *et al.* (2002) that dietary vitamin C and vitamin E act synergistically in laying hens at high ambient temperature.

In tibia bone breaking strength the vitamin C supplemented group showed 37.16 kg, followed by 33.52 kg of the combined vitamins C and E supplemented group and 29.22 kg of the vitamin E supplemented group. It differed significantly ($P<0.05$). Tibia bone weight and length were not affected by vitamin C and/or E supplementation (Table 4). Researchers reported that vitamin C had a positive effect on bone parameters, particularly bone breaking strength. This result was similar to Weiser *et al.* (1988) that reported improvement in bone weight, matrix, and ash in

Table 4. Effects of dietary vitamins C and E on tibia bone measurements under heat stress at 32 ℃ over 3 weeks[1]

Treatment	Body Weight	Tibia		
	g	Weight, g	Length, cm	Breaking Strength, kg
Basal Diet	3762 ± 343	22.81 ± 0.77	12.2 ± 0.4	29.91 ± 2.12[a]
B.D.+ vit.C[2]	3890 ± 254	23.44 ± 0.87	12.2 ± 0.2	37.16 ± 2.91[b]
B.D.+ vit.E[3]	3889 ± 391	22.52 ± 0.03	12.2 ± 0.2	29.22 ± 7.86[a]
B.D.+ C + E[4]	4049 ± 451	23.59 ± 0.67	12.3 ± 0.1	33.52 ± .75[ab]

[a,b] Means in a column with no common superscripts are significantly different ($P<0.05$).
[1] Data are mean values± standard deviation of eight samples.
[2] Basal Diet + vitamin C (200 mg/kg).
[3] Basal Diet + vitamin E (250 mg/kg).
[4] Basal Diet + vitamin C (200 mg/kg) + vitamin E (250 mg/kg).

chicks fed different levels of vitamin C in conjunction with cholecalciferol.

The values of serum concentration of ascorbic acid were the highest in the birds fed the vitamin C supplemented diet at the level of 12.73 mg/ml, followed by 8.23 mg/ml of the vitamins C and E supplemented treatment ; whereas the higher values of serum vitamin E concentrations were in the vitamin E supplemented group and the vitamins C and E supplemented one at 8.20 mg/ml and 8.35 mg/ml, respectively($P<0.05$). The results were similar to Amakye-Anim *et al.*(2000) observing that serum concentrations of vitamin C ranged from 3~6 mg/ml in chickens fed vitamin C unsupplemented diets at 31 days of age and ranged from 10~14 mg/ml in birds fed vitamin C supplemented diet at the same age. The hens fed the basal diet showed the highest serum corticosterone level, a mean of 5.97 ng/ml, among the treatments ($P<0.05$) (Table 5). On the other hand, other researchers observed that serum concentrations of corticosterone ranged from 10~18 ng/ml in chickens fed vitamin C unsupplemented diets at 31 days of age and ranged from 7~13 ng/ml in birds fed vitamin C supplemented diet at the same age(Amakye-Anim *et al.*, 2000).

Table 5. Serum concentration of vitamin C, E, and corticosterone in broiler breeder hens under heat stress at 32 ℃[1]

Component	Basal Diet	B.D.+vit.C[2]	B.D.+ vit.E[3]	B.D.+ C + E[4]
Vitamin C, μg/ml	7.87 ± 1.82[a]	12.73 ± 1.80[b]	7.26 ± 0.92[a]	12.24 ± 1.22[b]
Vitamin E, μg/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 low with no common superscripts are significantly different ($P<0.05$).
[1] Data are mean values± standard deviation of three samples.
[2] Basal Diet + vitamin C (200 mg/kg).
[3] Basal Diet + vitamin E (250 mg/kg).
[4] Basal Diet + vitamin C (200 mg/kg) + vitamin E (250 mg/kg).

Table 6. Effects of dietary vitamin C & E on differential white blood cells under heat stress at room temperature 32℃[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.+ vit.C[2]	57.8 ± 5.9	54.3 ± 6.5	53.8 ± 5.6
	B.D.+ vit.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	64.5 ± 5.7	59.3 ± 4.8	31.0 ± 4.7
	B.D.+ vit.C[2]	64.5 ± 5.7	59.3 ± 4.8	36.0 ± 4.5
	B.D.+ vit.E[3]	64.5 ± 5.7	59.3 ± 4.8	31.4 ± 7.0
	B.D.+ C + E[4]	64.5 ± 5.7	59.3 ± 4.8	34.8 ± 2.4
H/L ratio	Basal Diet	2.3 ± 0.6	2.5 ± 1.2	1.9 ± 0.4
	B.D.+ vit.C[2]	1.7 ± 0.4	2.6 ± 0.2	1.5 ± 0.3
	B.D.+ vit.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] Data are mean values± standard deviation (n=5). No differences exist among groups.
Heterophils were 33.7 and 58.3% before and after the experiment, respectively.
Lymphocytes were 59.7 and 33.5% before and after the experiment, respectively.
H/L ratios were 0.6 and 1.9 before and after the experiment, respectively, on the average.
[2] Basal Diet + vitamin C (200 mg/kg)
[3] Basal Diet + vitamin E (250 mg/kg)
[4] Basal Diet + vitamin C (200 mg/kg) + vitamin E (250 mg/kg)

H/L ratios tended to be lower in the vitamins C and E supplemented group than the basal diet group during the first two weeks after heating (Table 6). High temperature elevated heterophils up to 64.5 % from 33.7 %, whereas reduced lymphocytes down to 29.5 % from 59.7 % in the basal diet group. There was no statistical difference among the groups ($P<0.05$).

Corticosteroid concentration in the blood and H/L ratios have been used as measures of environmental stress and physiological activity in chickens, and the H/L ratio appears to be a more reliable indicator to social stress than were the plasma corticosterone levels(Edens and Siegel, 1975 ; Siegel, 1980 ; Gross and Siegel, 1983). This study showed that hens fed the vitamins C and E supplemented diet had lower serum corticosterone levels and H/L ratios than those fed the basal diet. This result suggested that vitamins C and E alleviate environmental stresses, particularly heat stress.

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

적 요

본 연구에서는 사료 중 비타민 C(200 mg/kg)와 비타민 E(250 mg/kg)의 첨가가 heat stress하 육용종계의 난각 품질에 미치는 영향을 조사하였다. 83주령의 강제환우된 Ross 육용종계 160수를 공시하여 4처리 4반복 그리고 반복당 10수를 무작위로 개별 케이지에 수용하였다. 4처리구 중 대조구는 비타민 C와 E를 추가로 첨가하지 않았으며 시험구는 비타민 C 처리구, 비타민 E 처리구, 그리고 비타민 C/E 동시 처리구로 하였다. 실온 25 ℃에서 10일간의 적응기간 후에 32 ℃에서 3주간 실험하였다. 실험기간 중 산란율은 급속히 떨어졌으나 처리구간에 유의한 차이가 없었으나 난중, 비중, 난각두께, SWUSA, pucture force와 난각 파괴강도에서는 비타민 C/E 처리구가 대조구에 비해 유의하게 높았다($P<0.05$). 비타민 C 처리구는 경골 파괴강도에서 37.16 kg으로 대조구 및 비타민 E 처리구에 비해 통계적으로 유의하게 높았다($P<0.05$). 한편, 혈중 corticosterone 수준(5.97 ng/ml)은 대조구가 다른 시험구들보다 유의하게 높았다($P<0.05$). Heat stress는 백혈구 중 heterophil의 비율을 높이고 lymphocyte의 비율은 낮추어 H/L ratio를 변화시켰다. 시험구의 H/L ratio는 대조구보다 낮았으나 통계적으로 유의한 차이를 나타내지 않았다. 결론적으로 사료 중 비타민 C(200 mg/kg)와 비타민 E(250 mg/kg)의 첨가는 육용종계에서 heat stress에 의한 영향을 경감하여 난각품질 및 경골강도의 약화를 방지한다고 할 수 있겠다.

Key words : vitamin C, vitamin E, egg shell quality, broiler breeder hens, heat stress, H/L ratios

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