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Vitamins C and E affect plasma metabolites and production performance of layer chickens *(Gallus gallus domesticus)* under condition of high ambient temperature and humidity

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Abstract

This study was carried out to investigate the effects of vitamins C and E on some plasma metabolites and production performance of layer chickens reared under hot tropical climate. 720 White Leghorn (L_{33}) layer chickens and 39 weeks old were divided into four groups of 180 birds. One group was fed with basal diet (control) and treatment groups were fed with basal diet supplemented with 150 mg of vitamin C/kg of diet, 150 mg of vitamin E/kg of diet, while the last group was supplemented with 150 mg of vitamin C/kg of diet plus 150 mg of vitamin E/kg of diet. Separately or as a combination, supplemental vitamin C and E decreased plasma concentrations of cholesterol, glucose and creatine phosphokinase ($P<0.001$) compared to control. There were no significant ($P>0.05$) effects of supplemental vitamin C and E on plasma metabolites of alanine phosphatase and aspartate transaminase. The single supplementation of vitamin E and its combination with vitamin C considerably increased the plasma metabolite of protein ($P<0.01$). Egg/bird were significantly ($P<0.05$) higher in all treatment groups compared to control, but *P* value was highest in vitamin E treated group. The laying index showed a significant ($P<0.05$) increase in all treatment groups compared to control. Similarly, feed consumption and conversion were significantly ($P<0.05$) different in treatment groups compared to control.

It is concluded that supplementation of vitamins C and E maintained the stability of some plasma metabolites concentration, thereby, sustained production performance and facilitated adaptation of chicken to stressful hot-humid condition.

Keywords: layer chicken, high temperature and humidity, vitamin C, vitamin E, plasma metabolites, production performance

Zusammenfassung

Einfluss von Vitamin C und E auf Plasmametaboliten sowie Produktionsmerkmale bei Legehennen unter Bedingungen hoher Temperatur und Luftfeuchtigkeit

Untersucht wurde der Einfluss von Vitamin C und E Futterzusätzen auf einige Plasmametaboliten und Produktionsmerkmale von Legehennen die unter tropischen

Bedingungen gehalten wurden. 720 Weiße Leghornhennen im Alter von 39 Wochen wurden in vier Gruppen mit je 180 Tieren aufgeteilt. Neben der Kontrollgruppe erhielten die anderen Gruppen Futterergänzungen von jeweils 150 mg Vitamin C/kg, 150 mg Vitamin E/kg bzw. 150 mg Vitamin C+150 mg Vitamin E/kg. Verglichen mit der Kontrollgruppe verminderte sich signifikant die Plasmakonzentration von Cholesterol, Glukose und Kreatinphosphokinase. Kein signifikanter Einfluss ergab sich bei Vitamin C und E Ergänzungen auf die Alaninphosphatase und Aspart Transaminase. Beträchtlich erhöhte sich sowohl bei Vitamin E als auch in Kombination mit Vitamin C die Eiweiß Plasmametaboliten. Die Legeleistung und der Legeindex waren bei allen Versuchsgruppen signifikant höher als bei der Kontrolle. Futterverbrauch und Futterverwertung unterschieden sich ebenfalls signifikant von der Kontrollgruppe. Es wird geschlussfolgert, dass eine Ergänzung mit Vitamin C und E die Konzentration einiger Plamametaboliten verbessert und damit ein Erhalt der Leistungsfähigkeit und die Anpassung an tropischen Bedingungen erleichtert werden.

Schlüsselwörter: Legehennen, hohe Temperaturen, Luftfeuchtigkeit, Vitamin C, Vitamin E, Plasmametaboliten, Produktionsleistungen

Introduction

With increased global warming at the turn of this millennium, heat stress has been the major preoccupation of livestock farming, particularly in the poultry sector (DAGHIR 2009). This is because poultry are particularly sensitive to fluctuations in ambient temperature (AT). Heat stress interferes with the birds comfort and suppresses production efficiency. In birds, high AT leads to increased endogenous heat production, since convective transfer of heat is the major thermo-regulatory mechanism of chickens and depends on movement of air by natural or fan-powered ventilation (SHANE 2005). As a response birds have to make major thermo-regulatory adaptations to prevent death from heat exhaustion. The result is that the full genetic potential of layer is often compromised (HOLIK 2009).The combination of high AT and relative humidity (RH) distress continues to be one of the major environmental perturbations reducing bird's performance; it induces hyperglycemia, reduces plasma protein concentration, and increases cholesterol (DONKOH 1989, SEYREK *et al*. 2004). OZCELIK and OZBEY (2004) and KHAN and SARDAR (2005) reported that heat stress reduced plasma alkaline phosphatase, aspartate amino transaminase and alanine transaminase concentrations. Researchers have tried to mitigate the effect of heat stress by changing the environment and diets of laying chickens. Nutritional strategy during heat period is based on diet balancing in order to cover the needs of stressed birds for ideal amino acids (protein), energy and electrolytes (BALNAVE 2004, DAGHIR 2009). For this purpose, vitamin C and vitamin E are used in the poultry diet because of their anti-oxidant properties in the neutralization of the free radicals generated during heat stress (RAMNATH *et al*. 2008). Poultry are renal synthesizers of vitamin C, but its quantity becomes insufficient under praxis conditions as a result of increased rate of usage in combating the free radicals thus generated (MAURICE *et al*. 2002). Vitamin E has been reported in the participation of the supply of egg precursors in plasma, while at the same time decreasing serum ACTH concentration (CIFTCI *et al*. 2005). KEVIN (1982) showed that dietary supplementation of vitamin E increased the fertility of poultry, normal testicular

functions of cockerels, layability of laying chickens as well as the hatchability of breeder eggs. Furthermore, the supplementation of vitamin C and E have been reported to increase serum concentration of total protein, but decrease corticosterone, glucose, and cholesterol concentrations in Japanese quails exposed to 33 °C (SAHIN *et al*. 2003b). Vitamin C has been demonstrated to be a powerful antioxidant that acts through a two way mechanism, that is, through its conversion to L-dehydroascorbic acid, a particularly inert radical, this reaction is reversible and the interconversion of these molecules forms a redox system which is the basic physiology of their actions, because both show vitamin C activity (COMBS 1992, YILDIZ *et al*. 2009). The other route, is the formation of an ascorbate radical that destroys free radicals generated by oxygen, which includes the hydroxyl (OH^*), mono-oxygen (O^*) and the superoxides (O^{2*}) and also in the transfer of radical equivalents from lipid phases to aqueous compartment. In realizing this function, the vitamin enters into a synergistic action with other protective enzymes such as, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase ($GSHP_x$). In its scavenging function for free radicals generated in the cell membranes, the vitamin helps in the conversion of the oxidized form of vitamin E to its stable form through a non-enzymatic reaction. Similarly, vitamin E has been demonstrated to be an antioxidant that scavenges the free radicals generated in cell membranes that participates in tissular degeneration (BOLLINGIER-LEE *et al*. 1999). The vitamin participates in a tripartite interaction together with selenium, an integral chemical complex of the enzyme $GSHP_x$ as protagonists, while the poly unsaturated fatty acids (PUFA) serve as the antagonist (ROTRUCK *et al*. 1972). The synergic effects between these two vitamins are particularly efficient for reducing production of reactive oxygen species in both aqueous and lipid phase of the cell membrane (CIFTCI *et al*. 2005). Because radical reactions are exergonic, they contribute to the failure of thermoregulatory process in hyperthermia observed during heat stress (MUJAHID *et al*. 2005).

The aim of this study therefore, was to investigate the possible beneficial effects of dietary vitamin C and vitamin E supplementation on plasma metabolites and production performance of layer chickens reared under hot-humid climate and during the summer period.

Material and methods

Experimental site and meteorological data

The study was conducted at the poultry production unit of »Las casas II«, situated in Santa Clara; Villa Clara (22° 53´ N and 82° 02´ W), in Central province of Cuba, with an altitude between 90-100 m above sea level. Total precipitation during the study period was 327.2 cm, while average air velocity was 3.15 m/s.

The AT and RH inside and outside the pen were recorded daily throughout the experimental period at 09:00, 12:00, 15:00 and 18:00 h respectively. A standard ambient thermometer for AT and wet and dry-bulb hygrometer for RH were used. The wet and dry bulb values were recorded, and RH calculated using the depression factor as indicated in the manufacturer's manual. Both instruments from (Cocet, China), were obtained from a commercial medical equipment sales outlet in Panama City.

Experimental birds

A total of 720 and 39 weeks old commercial L_{33} layer chickens, were used as subjects for the experiment. The birds were randomly divided *in situ* within production pen into 4 groups of 180 each, and each group was further divided into four replicates of 45 birds, and three birds/cage of 4.1×4.1×4.3 m dimension. One group was fed with basal diet (control group) and treatment groups were fed with the basal diet supplemented with either 150 mg of l-ascorbic acid/kg of diet (vitamin C group), 150 mg of α-dl-tocopherol acetate/kg of diet (vitamin E group), while the last group was supplemented with 150 mg of l-ascorbic acid/kg of diet plus 150 mg of α-dl-tocopherol acetate/kg of diet (vitamin C+E group). Vitamin C and vitamin E used were from a commercial company (VMD, n.v./s.a, Arendonk, Belgium). Prior to the experiment the birds were duly dewormed and vaccinated according to UECAN (2002) specifications. In addition, specific gravity fecal flotation method with modified Sheather's solution (DAVID and LINDQUIST 1982) were employed to confirm the absence of helminthes in the birds before commencement of experiment. The birds were fed with a basal diet of 110 g/bird/day, while water was given *ad libitum*. Feed constituents and bromatological analysis of the basal diet are shown in (Table 1).

Table 1
Composition and calculated bromatological analysis of basal diet
Futterzusammensetzung

	Quantity, kg
Nutrients/constituents	
Maize	60.7
Soya cake	26.8
Vegetable oil	1.1
Calcium carbonate	9.17
Monocalcium phosphate	1.12
Monocalcium	0.07
Choline chloride	0.3
Sodium chloride	0.25
Pre-mix Vitamins[a)] and Minerals[b)]	0.30
DL-Methionine	0.19
Calculated analysis, kg	
EM, MJ/kg	11.5
CP, g	16.5
Lysine, g	0.96
Methionine+Cystine, g	3.65
Tryptophan, g	0.23
Threonine, g	0.70
Ca, g	3.52
P[a)], g	0.25
Na, g	0.15
Cl, g	0.13

Source: UEB feed factory, Ministry of Agriculture, Villa Clara (2009), [a)]Vitamins supplement per (kg) of diet: Vitamin A, 12 000 UI; vitamin D_3, 2 500 UI; vitamin E, 5 UI; vitamin K_3, 4.5 mg; thymine, 1.5 mg; riboflavin, 4.20 mg; vitamin B_{12}, 12.2 µg; pyridoxine, 4 mg; pantothenic acid, 5 mg; nicotinic acid, 10 mg; folic acid, 0.5 mg; choline, 3 mg, [b)]Mineral supplement: Magnesium, 56 mg; iron, 20 mg; copper, 10 mg; zinc, 50 mg; cobalt, 125 mg; iodine, 0.08 mg, P[a)]=Available phosphorus

The basal diet contained 2 850 kcal/kg metabolic energy (ME) and 20.1 % crude protein (CP), 4.0 % Ca, 0.60 % P and 12.6 % ash, this was calculated to slightly exceed the nutrient requirements recommended by the National Research Council (NRC 1994). Blood samples were taken through venopuncture of the brachial vein into previously sterilized test tubes with heparin, gently depositing 5 mL of blood into test tubes, the samples were later centrifuged at 3 500 g for 15 minutes, in order to obtain blood plasma, these were later stored at −10 °C until analysis were carried out. Plasma metabolites: Plasma metabolites diagnosis was carried out at the beginning of the experiment, two weeks into the experiment, and at week four coinciding with the end of the experiment. Metabolites concentrations were measured spectrophotometrically (HOLLANDS and LOGAN 1966), with a biochemical analyzer SP-9 equipment (PYE UNICAM, Germany). All metabolites were analysed with reagents from Helfa® Diagnósticos and according to manufacturer's specifications. Egg/bird: This was carried out daily by dividing the numbers of eggs produced by the numbers of birds in each group throughout the experimental period. Laying index: This was conducted weekly for each group using the following formula:

$$LI = \frac{TN_h}{TN_g} \times 100 \quad (1)$$

Where LI is the Laying index, TN_h is the total numbers of eggs and TN_g is the total numbers of birds

Feed consumption: This parameter was conducted on a daily basis through the subtraction of the left over feed in each group from the amount previously supplied using a standard measuring balance (Salter, England) with maximum calibration of 5 kg, and a precision of 0.5 g. Feed conversion: This index was calculated on a weekly basis for each group. This was expressed as the amount of feed consumed (KG) in order to produce 10 eggs. Mortality: This was calculated as the percentage expression of the subtraction of birds that died in each group at the end of the experiment, from the allotted numbers at the beginning of the experiment

Statistical Analyses

The PC STATISTICA 8.0 package was used. Data for ambient temperature and relative humidity were analysed using Student's t-test and expressed as mean standard error of the mean (Mean±SEM). *P* value less than 0.05 were considered significant. Data for plasma metabolites and production performance were subjected to a one-way analysis of variance (Anova), through the general-linear-models procedure of the Statistical Analysis System (SAS 1985). Duncan's post-hoc test was used to identify means that differed at $P<0.05$ (DUNCAN 1955).

Results

The meteorological data during the experimental period are presented in (Table 2). Throughout the study period, the AT outside the pen was higher ($P<0.05$) than inside, while the values recorded for the RH inside the pen were significantly ($P<0.05$) higher than outside.

Table 2
Meteorological data of ambient temperature and relative humidity during the experimental period
Meteorologische Daten für Temperatur und Luftfeuchtigkeit während der Versuchsperiode

Hour	Ambient Temperature, °C			Relative Humidity, %		
	Out	In	Mean±SEM	Out	In	Mean±SEM
09:00 a.m.	31.8[a]	29.4[b]	± 0.527	85.6[b]	88.6[a]	± 1.729
12:00 noon.	35.6[a]	33.3[b]	± 0.421	75.4[b]	81.4[a]	± 1.908
03:00 p.m.	35.8[a]	34.0[b]	± 0.848	78.6[a]	79.6[a]	± 2.529
06:00 p.m.	29.0[a]	28.5[a]	± 0.619	86.3[a]	88.9[a]	± 1.972
Mean	33.0[a]	31.3[b]	± 0.583	81.5[b]	84.6[a]	± 1.312

Out Outside the pen, In Inside the pen, Mean±SEM Mean standard error of the mean between values recorded outside and inside the pen. For each parameter, mean values with different superscript alphabets along the same row are significantly ($P<0.05$) different.

Plasma concentrations of cholesterol and creatine phosphokinase were significantly ($P<0.001$) reduced in all treated groups compared to control (Table 3). The glucose level was significantly ($P<0.001$) lower in vitamin E and vitamin C+E treated groups compared to control, while in vitamin C treated group the level of reduction was ($P<0.05$) compared to control. There were no significant ($P>0.05$) effects of supplemental vitamin C and E on plasma metabolites of alanine phosphatase and aspartate transaminase. Although there was no significant ($P>0.05$) difference in the overall mean of plasma protein between experimental groups. However, the groups supplemented with vitamin E and vitamin C+E were considerably higher ($P<0.01$) by the fourth week of the experiment when compared with vitamin C and control groups respectively. Production performance parameters were presented in (Table 4). Egg/bird were significantly ($P<0.05$) higher in all treatment group when compared to control, but *P* value was particularly highest in vitamin E treated group. Overall egg/bird increased consistently in all groups from highly significant ($P<0.01$) difference in week 1 to very highly significant ($P<0.001$) difference from weeks 2 to 4. In the same vein, the laying index showed a significant ($P<0.05$) in all treatment groups when compared to control, and *P* value was particularly highest in vitamin E treated group. Overall laying index increased consistently in all groups from highly significant ($P<0.01$) difference in week 1 to very highly significant ($P<0.001$) difference from weeks 2 to 4. Feed consumption and conversion were significantly ($P<0.05$) different in vitamin C, vitamin E and vitamin C+E supplemented groups when compared to control group. Viability/mortality was not affected by vitamin C, vitamin E and vitamin C+E supplemented groups when compared to control ($P>0.05$).

Table 3
Impact vitamins C and E on some plasma metabolites of White Leghorn (L_{33}) layer chickens reared at high ambient temperature and humidity (n=80)
Wirkung der Vitamin E und C Zugaben auf einige Plasmametaboliten

Parameters in weeks	Treatments Vitamin-C	Vitamin-E	Vitamin-C+E	Control	SEM
Cholesterol, mmol/L					
1	4.39[a]	4.30[a]	4.34[a]	4.32[a]	± 0.074
2	4.53[b]	4.39[b]	4.42[b]	4.88[a]	± 0.047***
4	4.54[b]	4.41[b]	4.44[b]	6.06[a]	± 0.065***
$\bar{x}$	4.48[b]	4.37[b]	4.40[b]	5.09[a]	± 0.059***
Glucose, mmol/L					
1	6.33[a]	6.37[a]	6.34[a]	6.35[a]	± 0.040
2	6.70[b]	6.39[c]	6.56[bc]	7.51[a]	± 0.065***
4	6.86[b]	6.49[c]	6.52[c]	8.16[a]	± 0.076***
$\bar{x}$	6.63[b]	6.41[c]	6.47[bc]	7.34[a]	± 0.062***
Alkaline phosphatase, u/L					
1	31.41[a]	31.47[a]	31.61[a]	31.51[a]	± 0.086
2	31.21[a]	31.16[a]	31.21[a]	31.22[a]	± 0.158
4	31.19[a]	31.16[a]	31.19[a]	31.16[a]	± 0.146
$\bar{x}$	31.27[a]	31.26[a]	31.33[a]	31.30[a]	± 0.078
Aspartate transaminase, u/L					
1	33.90[a]	34.05[a]	34.25[a]	34.30[a]	± 0.413
2	34.04[a]	34.06[a]	34.09[a]	34.28[a]	± 0.197
4	34.02[a]	34.04[a]	33.95[a]	34.24[a]	± 0.203
$\bar{x}$	33.98[a]	34.05[a]	34.10[a]	34.27[a]	± 0.164
Creatine phosphokinase, u/L					
1	141.61[a]	141.43[a]	141.31[a]	141.49[a]	± 0.108
2	142.33[b]	143.43[b]	143.01[b]	147.88[a]	± 0.401***
4	142.58[b]	143.52[b]	143.15[b]	153.24[a]	± 0.571***
$\bar{x}$	142.17[b]	142.79[b]	142.49[b]	147.54[a]	± 0.398***
Total protein, g/L					
1	51.95[a]	52.02[a]	51.62[a]	52.22[a]	± 0.419
2	55.91[a]	55.72[a]	54.38[a]	54.52[a]	± 1.362
4	57.29[b]	63.76[a]	62.38[a]	58.47[b]	± 1.293**
$\bar{x}$	55.05[a]	57.17[a]	56.12[a]	55.07[a]	± 0.798

SEM Standard error of the mean. Means values with different superscripts alphabets along the same row are significantly different. Level of significance: *(P<0.05), **(P<0.01), ***(P<0.001). Results are expressed as means ± standard deviations.

Table 4
Impact of vitamins C and E on production performance of White Leghorn layer chickens (L_{33}) reared at high ambient temperature and humidity (n=80)
Wirkung der Vitamin C und E Zugaben auf Produktionsmerkmale

Parameters in weeks	Treatments Vitamin-C	Vitamin-E	Vitamin-C+E	Control	SEM
Egg/bird					
1	5.92[b]	5.96[ab]	6.03[a]	5.82 [c]	± 0.007[**]
2	6.21[a]	6.15[a]	6.16[a]	5.73 [b]	± 0.006[***]
3	6.16[a]	6.27[a]	6.18[a]	5.63 [b]	± 0.009[***]
4	6.07[b]	6.33[a]	6.16[b]	5.53 [c]	± 0.009[***]
$\bar{x}$	24.26[b]	24.60[a]	24.46[ab]	22.67 [c]	± 0.017[***]
Laying index, %					
1	84.60[b]	85.08[ab]	86.11[a]	83.09 [c]	± 0.094[**]
2	88.66[a]	87.79[a]	87.94[a]	81.90 [b]	± 0.083[***]
3	88.03[a]	89.63[a]	88.33[a]	80.40 [b]	± 0.123[***]
4	86.76[b]	90.42[a]	87.97[b]	78.93 [c]	± 0.130[***]
$\bar{x}$	86.65[b]	87.86[a]	87.34[ab]	80.97 [c]	± 0.061[***]
Feed consumption/kg/bird					
1	0.755[a]	0.759[a]	0.763[a]	0.745 [a]	± 0.001
2	0.766[a]	0.765[a]	0.763[a]	0.738 [b]	± 0.002[*]
3	0.762[a]	0.759[a]	0.749[a]	0.733 [b]	± 0.001[**]
4	0.767[a]	0.764[a]	0.760[a]	0.739 [b]	± 0.001[*]
$\bar{x}$	3.037[a]	3.034[a]	3.027[a]	2.950 [b]	± 0.004[**]
Conversion/kg of feed/10 eggs					
1	1.276[a]	1.274[a]	1.267[a]	1.281 [a]	± 0.003
2	1.234[b]	1.246[b]	1.239[b]	1.287 [b]	± 0.002[**]
3	1.237[b]	1.209[b]	1.211 [b]	1.302 [a]	± 0.002[***]
4	1.262[b]	1.207[c]	1.235 [bc]	1.337 [d]	± 0.003[***]
$\bar{x}$	1.252[b]	1.234[b]	1.238 [b]	1.301 [a]	± 0.001[***]
Mortality, %					
1	99.44.[a]	99.44.[a]	100.00.[a]	100.00.[a]	± 0.088
2	100.00.[a]	100.00.[a]	100.00.[a]	100.00 [a]	± 0.000
3	100.00.[a]	100.00.[a]	98.89 [a]	99.44 [a]	± 0.095
4	99.44.[a]	100.00.[a]	100.00 [a]	99.44 [a]	± 0.088
$\bar{x}$	98.89.[a]	99.44.[a]	98.89 [a]	98.89 [a]	± 0.139

SEM Standard error of the mean. Means values with different superscripts alphabets along the same row are significantly different. Level of significance: *(P<0.05), **(P<0.01), ***(P<0.001). Results are expressed as means ± standard deviations.

Discussion

In this study, the AT both inside and outside the pen during the study period were higher than the recommended normothermia zone of 22-28 °C (DONKOH 1989) or 18-24 °C (HOLIK 2009) established for poultry in the tropical regions. The combination of 31.3 °C and 33.0 °C recorded for AT with 84.6 % and 81.5 % recorded for RH were extrapolated using the mathematical model established by (TAO and XIN 2003) to give a temperature humidity index (THI) of 85.5, a value above the THI threshold of 70, established for poultry (TAO and XIN 2003). This is a clear indication that the layer chickens were subjected to heat stress. NALINI *et al*. (2008) observed that higher temperatures resulted in greater variations in hormones and interrelated analytes

than the lower temperatures. The authors reported that at 42-45 °C, serum corticosterone, growth hormone, glucagon, testosterone, uric acid, creatine, urea, glucose, cholesterol, triglycerides, free fatty acids, AST, ALT, lipase and amylase increased significantly ($P<0.05$) from respective control mean values. It is well documented that heat stress overtaxes the thermoregulatory mechanism of birds, which leads to alteration of biological function and a shift in biological resources as response solution (MANTEUFFEL 2002). The mechanism of this alteration has been reported to be through increased generation of free radicals at the cell level. Several authors have documented (ALTAN *et al*. 2003, LIN *et al*. 2005, IMIK *et al*. 2009) that free radical generation affects blood serum metabolites of AST, ALP, CPK, TP, cholesterol and glucose which is manifested in bird's adaptation response through decreased production performance. VANDENDRIESSCHE and ARNOUTS (2005) confirmed this in the results of their trial experiment, when they reported that the blood parameters of AST, ALP and CPK can be used to make a distinction between healthy and systemic diseased animals. Vitamin C antioxidant property has long been documented (GERMAN and TRABER 2001). Likewise, it has long been recognized that tocopherol possess antioxidant activity (EID *et al*. 2008). In the present study, vitamins C and E supplementation improved the stability of serum metabolites of L_{33} laying chickens reared under high ambient temperature and humidity. Decreased cholesterol concentrations found in the present study were in agreement with previous report (SAHIN *et al*. 2003a). Similarly, TAKEDA and HARA (1985) reported that vitamin C supplementation decreased serum enzymes levels. Furthermore, the same authors reported that the activities of blood enzymes (ALP, SGPT and SGOT) in groups of layers supplemented with vitamin C was lower ($P<0.01$) than those of groups without the vitamin. According to BHATTI *et al*. (2003) and BHATTI and DIL (2005), alteration in serum enzymes activity under stress conditions occur due to malfunctioning of liver, as degenerating and necrotic cells leak enzymes from cytoplasm. Our observation in this study is in agreement with that reported by OZCELIK and OZBEY (2004), who observed no change in blood parameters of AST and ALT, after a 2 weeks exposure of Japanese quails to two different AT of 18-24 °C and 35 °C respectively. Likewise, OZBEY *et al*. (2004) and SEYREK *et al*. (2004) reported an increase in plasma concentrations of Cholesterol and glucose in control group compared to the treated groups in Japanese quails exposed to heat stress at 35 °C and 34 °C respectively. Results of the present study showed similar trends for effects of vitamin C and vitamin E, as evidence that serum glucose, and cholesterol concentrations decreased, while protein concentrations increased by supplemental dietary vitamin C and E. SAHIN *et al*. (2002a) reported that vitamin C and E supplementation increased plasma protein concentration while markedly decreased blood ACTH, glucose and cholesterol concentrations in heat-stressed (34 °C) Japanese quails. Similarly, KUTLU and FORBES (1993) reported that vitamin C supplementation increased plasma protein concentration while markedly decreased blood glucose and cholesterol concentrations in heat-stressed (36 °C) broilers. Increases in concentrations of glucose may be attributed to induced glucocorticoid secretion which increases glucogenesis. Glucocorticoids are well known not only as hormones essential for maintaining life and normal growth, but also as stress hormones. Dietary vitamin C may reverse these changes, probably by reducing the secretion or synthesis of glucocorticoids (MCDOWELL 1989). Overall antioxidant potential has been reported to possibly be more efficient and crucial than single antioxidant nutrients (GERMAN and TRABER 2001). Feed conversion in layer chickens is subject to marked

fluctuations because of seasonal as well as ambient temperature changes. Several studies have reported that high temperatures reduce the efficiency of utilizing feed energy for productive purposes (OZCELIK and OZBEY 2004, SHANE 2005, DAGHIR 2009). Layers not only eat less at high temperature, but also produce less per unit of intake, especially at temperatures above 30 °C. In this experiment, the single or combined dietary supplementation with vitamins C and E of laying chickens exposed to summer tropical hot-humid climate have significantly improved production performances of feed consumption, conversion and egg/bird/day. Additions of vitamin E alone into diets appeared to be more beneficial for laying hens during heat stress. Probably, due to its concurrent function as fertility factor (SAHIN *et al*. 2002b). Vitamin E supplementation of diets containing high amounts of polyunsaturated fatty acids may prevent feed oxidation and may contribute to egg formation. These beneficial protective effects of vitamins were evidenced by increases of egg/bird/day; feed intake and efficiency in treatment groups in comparison to control chickens. This study is in agreement with the findings of SAHIN and KUCUK (2001), who reported that a combination of 200 mg of vitamin C and 250 mg of vitamin E provides the greatest performance in Japanese quails reared under heat stress, and that such a combination can be considered as a protective management practice in a poultry diet, ameliorating the detrimental effects of heat stress. Likewise, CIFTCI *et al*. (2005), reported that vitamin E can alleviate the depression in egg production in heat stressed laying chickens. These authors reported that the combination of vitamins C and E can attenuate the heat induced oxidative damage at the cell level. They concluded that their positive effects were evidenced by increased growth performances, egg production and improvement of egg qualities in comparison to non-supplemented birds.

Because of stability in the concentration of some plasma metabolites resulted by oral vitamins C and E supplementation; birds could remain healthy, maintained production performance and were acclimated to the heat stressor quicker than untreated group. In other words, vitamins C and E treatment facilitates adaptation of the chicken to stressful hot-humid condition.

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