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Impact of heat stress on egg quality in layer hens supplemented with l-ascorbic acid and dl-tocopherol acetate

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ABSTRACT

The aim of the experiment was to investigate the effects of heat stress on egg quality profile in layer hens supplemented with vitamins C and E. A total of 720 L_{33} layer hens at 39 weeks old, were divided into four groups of 180 birds. One group was fed a basal diet (control) and treatment groups were fed a basal diet supplemented with 150 mg of l-ascorbic acid/kg of diet (Vit. C group), 150 mg of α-dl-tocopherol acetate/kg of diet (Vit. 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 (Vit C + E group). Egg, albumen and eggshell weights were higher ($P<0.05$) and ($P<0.005$) in vitamins E and C + E groups when compared to vitamin C and control groups. Egg yolk was higher ($P<0.05$), ($P<0.005$) and ($P<0.0005$) in the vitamin C + E group, compared to the vitamin E and vitamin C treated groups and control respectively. Egg shell thickness, egg resistance and specific gravity showed ($P<0.05$) and ($P<0.005$) in the vitamins C + E group, compared to the vitamin C and E groups, and control. Haugh Unit was higher ($P<0.05$) and ($P<0.005$) in the vitamins C + E treated group compared to vitamin C and E treated groups, and control. The results suggest that the supplementation of antioxidant vitamins had a beneficial effect on egg quality in heat stressed layer hens.

Key words: heat stress, vitamin C, vitamin E, egg quality, layer hens

Introduction

Today a large percentage of the world's poultry population is located in regions where heat stress is a major management problem at some particular moments of the bird's productive lives. Farm animals also have a known zone of thermal comfort (ZTC) that primarily depends on the species, the physiological status of the animals, the relative humidity, velocity of ambient air and the degree of solar radiation (ANONYM., 1981).

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Economic losses are incurred by the poultry industry because farm animals are raised in places and seasons where temperature conditions venture outside the ZTC. Heat stress results from a negative balance between the net amount of energy flowing from the animal to its surrounding environment and the amount of heat energy produced by the animal. This imbalance is induced by changes in a combination of environmental factors (e.g. sunlight, thermal radiation and air temperature), animal properties (e.g. rate of metabolism and moisture loss) and thermoregulatory mechanisms such as conduction, radiation, convection, and evaporation. The bird's ability to dissipate heat during heat stress is compromised, making excessive heat production potentially life threatening. Thus, live weight, feed efficiency, egg production, egg quality, eggshell quality, fertility, hatchability and survival are affected (WIERNUSZ, 1998; ZAVIEZO, 1999). There are several environmental factors that affect layer chickens in production, but the most debilitating is heat stress, which affects production parameters, especially since birds are sensitive to heat waves because of their feather covering and lack of sweat glands, which makes heat dissipation difficult (ESTRADA-PAREJA et al., 2007). High temperatures, especially when coupled with high humidity, impose severe stress on birds and lead to reduced performance. Although a great deal of knowledge has been accumulated concerning the responses of poultry to high ambient temperatures, the role of RH (relative humidity) in intensifying or modifying these responses has received little attention and more particularly, air velocity (SIMMONS et al., 2003). Relative humidity is rarely included as an experimental variable or even measured for information purposes. Such information is important because in poultry-producing regions high temperatures can often be accompanied by a range of RH, which can markedly affect the degree of heat stress experienced by the birds (BALNAVE, 2004). ROMIJN and LOKHORST (1961) reported that the detrimental effects of high temperatures were more pronounced at higher RH. However, it was TAO and XIN (2003) who for the first time adapted a temperature humidity index (THI) for use with poultry, using wind speed as a variable, and called this index the temperature-humidity-velocity index (THVI) (Table 1). They also established several stages of thermal comfort values such as: normal ≤70, alert from 70 to 75, danger values are those from 76 to 81, and emergency values were ≥82, based on variations in the bird's body temperature, while employing the following mathematical model:

$$THVI = (0.85 \times DBT + 0.15 \times WBT) \times V^{-0.058}$$

THI = Temperature, Relative Humidity Index and Air Velocity;

DBT = Dry bulb temperature (ºC);

WBT = Wet bulb temperature (ºC).

V = Air velocity.

Thus, 70 was established as the standard threshold value for THVI in poultry.

Table 1. Temperature and humidity index values related to heat stress safety

Temperature °C \ Relative Humidity (%)	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
20	63	63	63	64	64	64	64	65	65	65	66	66	66	66	67	67	67	67	68	68
22	64	65	65	66	66	66	67	67	67	68	68	69	69	69	70	70	70	71	71	72
24	66	67	67	68	68	69	69	70	70	70	71	71	72	72	73	73	74	74	75	75
26	68	69	69	70	70	71	71	72	73	73	74	74	75	75	76	77	77	78	78	79
28	70	70	71	72	72	73	74	74	75	76	76	77	78	78	79	80	80	81	82	82
30	71	72	73	74	74	75	76	77	78	78	79	80	81	81	82	83	84	84	85	86
32	73	74	75	76	77	77	78	79	80	81	82	83	84	84	85	86	87	88	89	90
34	75	76	77	78	79	80	81	82	83	84	84	85	86	87	88	89	90	91	92	93
36	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	93	94	95	96	97
38	78	79	81	82	83	84	85	86	88	89	90	91	92	93	95	96	97	98	99	100

Source: (USDC-ESSA, 1970) as modified by (TAO and XIN, 2003).

Over the past two decades there has been a great deal of research and development in ways and means of reducing heat stress in birds subjected to high temperatures. The results obtained from these investigations have suggested the early acclimation of birds (McDONALD et al., 1990), nutritional manipulation and improved modern housing (DAGHIR, 2009). Housing and ventilation equipment are two of the most effective means of reducing heat stress, nevertheless the cost of building and maintenance is too high. This has led to seeking alternative cost-effective ways of combating heat stress, without creating additional stress in birds. Different authors have recommended, amongst other measures to mitigate the deleterious effect of heat, the supplementation of multivitamins and minerals, especially in layer chickens. The benefits of vitamin C, vitamin E, potassium chloride, ammonium chloride, potassium sulphate and sodium bicarbonate in drinking water or feed during the hot period of the day have been reported (SAHIN and KUCUK, 2001; CIFTCI et al., 2005). 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). Although poultry are renal synthesizers of vitamin C, its quantity becomes insufficient during heat stress as a result of t he increased rate of usage in combating the free radicals thus generated. On the other hand, vitamin E has been reported to participate in the supply of egg precursors in the plasma, while at the same time decreasing serum ACTH concentration. Furthermore, KEVIN (1982) observed that dietary supplementation of vitamin E increased the fertility of poultry, the normal testicular function of cockerels, layability of laying hens as well as the hatchability of breeder eggs. The aim of this study, therefore, was to investigate the possible beneficial effects of dietary vitamin C and vitamin E supplementation on egg quality in laying hens subjected to heat stress.

Materials and methods

Experimental site. The study was performed at the poultry production unit of "Las Casas II", located at 5 ½ Km along the Santa Clara and Camajauni highway in the province of Villa Clara. Total precipitation during the study period was 327.2 mm, while average air velocity was 3.15 m/s.

Experimental birds and feed. A total of 720, 39 week old commercial L_{33} layer hens were used as subjects for the experiment. The birds were randomly divided into four groups of 180 each, and each group was further divided into four replicates of 45 birds. One group was fed a basal diet (control group) and the treatment groups were fed the basal diet supplemented with either 150 mg of l-ascorbic acid/kg of diet (Vit. C group), 150 mg of α-dl-tocopherol acetate/kg of diet (Vit. 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 (Vit. C + E group). The Vitamin C and vitamin E used were from a

commercial company (VMD, Arendonk, Belgium). The birds were fed 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 2. The basal diet contained 2850 kcal/kg metabolic energy (ME) and 20.1% crude protein (CP), 4.0% Ca, 0.60% P and 12.6% ash, which was calculated to slightly exceed the nutrient requirements recommended by the National Research Council (ANONYM., 1994).

Table 2. Composition and calculated bromatological analysis of basal diet.

Nutrients/constituents	Quantity (Kg)
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, 12000 UI; vitamin D_3, 2500 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.

Experimental procedures. The AT and RH were measured daily using a standard thermometer (Harris, England) with a 42 ºC calibration and a standard hygrometer (Cocet, China) with a 50 ºC calibration respectively. All egg quality parameters measured during the period were carried out twice weekly using standard procedures.

Statistical analyses. All data were analyzed by analysis of variance procedures (ANOVA) and Duncan multiple-range test (RABE-HESKETH and EVERITT, 2004) using the package Statistica 5.0 and results were considered significant when p values were less than 0.05.

Results

The observed THI values are shown in Table 3.

Table 3. Temperature and relative humidity index during the study period

Hour	Average	SD	Min	Max	Range
9	83.1	± 2.510	79.6	91.0	11.4
12	88.4	± 1.767	85.6	93.5	7.9
3	89.1	± 2.915	78.5	93.1	14.6
6	81.6	± 2.367	77.8	87.0	9.2
Mean	85.5	± 4.056	77.8	93.5	15.7

SD = Standard deviation

The minimum THI value of 77.8 was recorded at 6.00 pm, while the maximum value of 93.5 was recorded at 12.00 noon. The highest range value of 14.6 during the experimental period was recorded at 3.00 pm, corresponding to the hottest period of the day. The average THI value was 85.5 during the same period. External egg quality parameters are presented in Table 4.

Absolute egg weight, yolk weight, albumen weight and eggshell weight were higher in all treatment groups compared to the control. The value of ($P<0.05$) was observed in the vitamin E and vitamins C+ Egroups when compared to the vitamin C group. Although there was no significant ($P>0.05$) difference in the egg shape external quality index (Table. 5) in all experimental groups, however, eggshell thickness, eggshell resistance and egg specific density were significantly ($P<0.01$) and ($P<0.05$) in the vitamins C + E group and the vitamin C and E groups when compared to the control. The egg internal quality (Table 6) Haugh unit (HU) on the other hand showed a significant ($P<0.001$) and ($P<0.05$) difference in the vitamin E treated group when compared with the control and the vitamin C and vitamins C + E treated groups respectively. The yolk index showed no significant ($P>0.05$) difference in all groups, while the albumen index was significantly ($P<0.001$) lower in the control compared to the vitamin E and vitamins C+E treated groups, while showing ($P<0.05$) compared to the vitamin C treated group respectively.

Table 4. Egg absolute weight and its principal components during the study period (n = 80)

Treatment parameters in weeks	Experimental groups				SEM
	Vit-C	Vit-E	Vit-C+E	Control	
Egg weight (g)					
I	60.39 ^{b}	61.95 ^{a}	61.76 ^{a}	58.48 ^{c}	± 0.186***
II	60.78 ^{a}	61.93 ^{a}	61.99 ^{a}	57.60 ^{b}	± 0.309***
III	60.77 ^{b}	62.09 ^{b}	63.69 ^{a}	56.32 ^{c}	± 0.278***
IV	61.17 ^{b}	64.97 ^{a}	64.98 ^{a}	56.96 ^{c}	± 0.368***
$\overline{x}$	60.78 ^{b}	62.74 ^{a}	63.10 ^{a}	57.34 ^{c}	± 0.309***
Egg yolk weight (g)					
I	15.64 ^{ab}	15.35 ^{bc}	15.86 ^{a}	15.16 ^{c}	± 0.072**
II	15.40 ^{ab}	15.97 ^{a}	15.97 ^{a}	15.11 ^{b}	± 0.101**
III	15.29 ^{b}	15.54 ^{b}	16.24 ^{a}	14.66 ^{c}	± 0.101***
IV	15.83 ^{b}	16.59 ^{a}	16.59 ^{a}	14.76 ^{c}	± 0.106***
$\overline{x}$	15.54 ^{c}	15.86 ^{b}	16.16 ^{a}	14.92 ^{d}	± 0.100***
Egg albumen weight (g)					
I	39.81 ^{b}	41.36 ^{a}	40.60 ^{a}	38.17 ^{c}	± 0.136***
II	39.58 ^{a}	40.02 ^{a}	40.38 ^{a}	37.23 ^{b}	± 0.250***
III	39.75 ^{b}	40.98 ^{ab}	41.45 ^{ab}	36.18 ^{c}	± 0.184***
IV	39.60 ^{b}	42.40 ^{a}	42.12 ^{a}	36.90 ^{c}	± 0.266***
$\overline{x}$	39.68 ^{b}	41.09 ^{a}	41.14 ^{a}	37.12 ^{d}	± 0.224***
Eggshell weight (g)					
I	4.94 ^{b}	5.25 ^{a}	5.30 ^{a}	5.16 ^{ab}	± 0.045*
II	5.82 ^{a}	5.95 ^{a}	5.65 ^{a}	5.27 ^{b}	± 0.058***
III	5.73 ^{b}	5.97 ^{a}	6.00 ^{a}	5.48 ^{c}	± 0.038***
IV	5.75 ^{b}	5.99 ^{b}	6.27 ^{a}	5.30 ^{c}	± 0.048***
$\overline{x}$	5.56 ^{b}	5.79 ^{a}	5.80 ^{a}	5.30 ^{c}	± 0.058***

Means with different alphabet superscripts along the same row are significantly different. Level of significance: * = (P<0.05), ** = (P<0.01), *** = (P<0.001).

Table 5. Egg external quality and index during the experimental period (n = 80)

Treatment Parameters in weeks	Experimental groups				SEM
	Vit-C	Vit-E	Vit-C+E	Control	
Eggshell thickness (mm)					
1	0.241 ^{a}	0.244 ^{a}	0.248 ^{a}	0.238 ^{a}	± 0.004
2	0.298 ^{c}	0.347 ^{b}	0.403 ^{a}	0.256 ^{d}	± 0.004***
3	0.317 ^{c}	0.344 ^{b}	0.411 ^{a}	0.287 ^{d}	± 0.004***
4	0.364 ^{b}	0.362 ^{b}	0.416 ^{a}	0.288 ^{c}	± 0.005***
$\bar{x}$	0.305 ^{b}	0.324 ^{b}	0.370 ^{a}	0.257 ^{c}	± 0.007***
Eggshell resistance (N)					
1	28.3 ^{a}	28.5 ^{a}	28.8 ^{a}	24.7 ^{b}	± 0.403***
2	33.3 ^{c}	37.7 ^{b}	42.5 ^{a}	27.6 ^{d}	± 0.366***
3	34.9 ^{c}	37.1 ^{b}	43.1 ^{a}	28.3 ^{d}	± 0.356***
4	39.0 ^{b}	38.9 ^{b}	43.8 ^{a}	28.3 ^{c}	± 0.469***
$\bar{x}$	33.8 ^{b}	35.5 ^{b}	39.5 ^{a}	27.2 ^{c}	± 0.620***
Egg specific gravity					
1	1.067 ^{a}	1.066 ^{b}	1.067 ^{a}	1.065 ^{b}	± 0.001***
2	1.068 ^{c}	1.069 ^{b}	1.070 ^{a}	1.067 ^{c}	± 0.001***
3	1.067 ^{c}	1.068 ^{b}	1.069 ^{a}	1.067 ^{c}	± 0.001***
4	1.069 ^{b}	1.068 ^{bc}	1.072 ^{a}	1.067 ^{c}	± 0.001***
$\bar{x}$	1.068 ^{b}	1.068 ^{b}	1.070 ^{a}	1.066 ^{c}	± 0.001***
Egg shape Index (%)					
1	74.65 ^{a}	73.05 ^{a}	74.00 ^{a}	74.10 ^{a}	± 0.613
2	72.95 ^{a}	73.00 ^{a}	72.90 ^{a}	72.15 ^{a}	± 0.660
3	74.05 ^{a}	74.30 ^{a}	73.40 ^{a}	71.95 ^{a}	± 0.621
4	74.60 ^{a}	74.60 ^{a}	73.30 ^{a}	71.10 ^{a}	± 0.655
$\bar{x}$	74.06 ^{a}	73.74 ^{a}	73.40 ^{a}	72.33 ^{a}	± 0.631

mm = millimetres, N = Newton. Means with different alphabet superscripts along the same row are significantly different. Level of significance: * = (P<0.05), ** = (P<0.01), *** = (P<0.001).

Table 6. Egg internal quality and index during the experimental period (n = 80)

Treatment Parameters in weeks	Experimental groups				SEM
	Vit-C	Vit-E	Vit-C+E	Control	
Haugh unit (HU)					
I	77.82 ^{b}	79.04 ^{ab}	79.67 ^{a}	76.26 ^{c}	± 0.246***
II	78.05 ^{ab}	79.23 ^{a}	78.98 ^{a}	76.66 ^{b}	± 0.255**
III	79.21 ^{a}	79.69 ^{a}	77.75 ^{b}	78.87 ^{ab}	± 0.201**
IV	78.32 ^{ab}	79.33 ^{a}	77.74 ^{b}	75.75 ^{c}	± 0.267***
$\overline{x}$	78.35 ^{b}	79.32 ^{a}	78.54 ^{b}	76.88 ^{c}	± 0.255***
Egg albumen Index (%)					
I	9.70 ^{b}	10.10 ^{ab}	10.30 ^{a}	9.00 ^{c}	± 0.083***
II	9.60 ^{b}	10.00 ^{a}	10.10 ^{a}	9.00 ^{c}	± 0.063***
III	9.00 ^{bc}	10.30 ^{a}	9.90 ^{ab}	9.50 ^{c}	± 0.066***
IV	10.00 ^{a}	10.40 ^{a}	10.30 ^{a}	8.90 ^{b}	± 0.077***
$\overline{x}$	9.80 ^{b}	10.10 ^{a}	10.20 ^{a}	9.10 ^{c}	± 0.074***
Egg yolk Index (%)					
I	55.60 ^{a}	55.20 ^{a}	55.70 ^{a}	55.60 ^{b}	± 0.052**
II	55.40 ^{b}	55.80 ^{a}	55.60 ^{ab}	55.70 ^{a}	± 0.052*
III	55.40 ^{a}	55.30 ^{a}	55.60 ^{a}	55.50 ^{a}	± 0.058
IV	55.60 ^{a}	55.60 ^{a}	55.80 ^{a}	55.40 ^{a}	± 0.059
$\overline{x}$	55.50 ^{a}	55.40 ^{a}	55.60 ^{a}	55.50 ^{a}	± 0.057

Means with different alphabet superscripts along the same row are significantly different Level of significance: * = (P<0.05), ** = (P<0.01), *** = (P<0.001).

Discussion

The values of 85.5 THI recorded in this study is above the threshold mark of 70 established for poultry. This indicated that the high AT and high RH acting on the birds during the study period predisposed the birds to heat stress, as evidenced by a decrease in production parameters. This finding is in agreement with the report by KARAMAN et al. (2007), who reported a decrease in egg production and dry matter intake (DMI) by using the daily total THI load as the driving variable of a mathematical model, with a threshold THI value of 70 during a one-day trial. They recorded a daily loss of egg production which ranged from 18.48 to 51.32 g/egg/hen/day, while the daily decrease of DMI ranged from 27.07 to 75.23 g/hen/day for a commercial egg production facility located in Tokat, Turkey, during the summer seasons of 2003 and 2004. Furthermore, the reduction in egg quality parameters recorded in our experiment is in agreement with SAHIN and KUCUK (2001), ROBERTS (2004) and CIFTCI et al. (2005) who reported that, exposure of Japanese quails

and laying hens to high ambient temperatures caused reduction in reproductive activities and egg quality respectively. The reduction in reproductive performance associated with heat stress is a well-known phenomenon in domestic birds (DAGHIR, 2009). This is probably due to the direct debilitating effect of high ambient temperature on ovarian function in the birds (ROZENBOIM et al., 2007). A possible mechanism for the reduction of ovarian function might be the reduction in blood flow to the ovary; a differential ovarian blood flow pattern was found in hens exposed to high ambient temperatures (MASHALY et al., 2004). The response of chickens at high temperatures differs with different relative humidity. It has been reported that a high temperature accompanied by high humidity is more detrimental to layer performance than a high temperature with low humidity. At the same time, constant high temperature of 30-32 °C is more deleterious to birds than cyclic or alternating temperatures of 30-32 °C by day and 25 °C by night, as it has been documented that the combination of the two stimulate the release of corticosteroids from the hypothalamus. Vitamin C is known to decrease the use of corticosteroids released during stress (SAHIN et al., 2003), thus playing an important role in response to stress. Single or combined dietary supplementation with vitamin C and vitamin E of laying hens exposed to heat stress in this study has significantly improved the egg quality parameters of egg weight, eggshell weight, albumen and yolk weight. Additions of vitamin E alone to the diet appeared to be more beneficial for laying hens during heat stress, probably due to its concurrent function as a fertility factor (KEVIN, 1982). 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, and the basic physiology of their actions, as both show vitamin C activity. The other route, is the formation of an ascorbate radical that destroys free radicals generated by oxygen, which includes hydroxyl ($OH^{\cdot}$), mono-oxygen ($O^{\cdot}$) and the superoxides ($O^{2\cdot}$) 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 antioxidant enzymes, such as: catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase ($GSHP_x$). PUTHPONGSIRIPORN et al. (2001) confirmed *in vitro* that the addition of vitamin C reduced the rate of proteolytic induction by hydrogen peroxide (H_2O_2) and the destruction of SOD. 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. In like manner, vitamin E has also been demonstrated to be an antioxidant that scavenges the free radicals generated in cell membranes that participate in tissular degeneration (BOLLINGIER-LEE et al., 1999; YARDIBI et al., 2009). The vitamin participates in a tripartite interaction together with selenium, an integral chemical complex of the enzyme GSHPx as protagonists, while poly unsaturated fatty acids serve as the antagonist (GSHPx) (ROTRUCK et al., 1973).

The synergic effects between these two vitamins are particularly efficient for reducing production of reactive oxygen species (ROS). Since radical reactions are exergonic, they contribute to the failure of the thermoregulatory process in hyperthermia observed during heat stress. Consequently, dietary supplementation of birds with vitamin C, vitamin E or a combination of these two anti-oxidant compounds can attenuate the deleterious heat-induced-oxidative stress. Vitamin E supplementation of diets containing high amounts of polyunsaturated fatty acids may prevent feed oxidation and may contribute to egg formation (BOLUKBASI et al., 2007). These beneficial protective effects of vitamins were evidenced by increases in Haugh unit, egg specific density, eggshell resistance, eggshell thickness and its indices in treatment groups in comparison to control chickens. The result in 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 poultry diet, ameliorating the detrimental effects of heat stress. In the same way, WHITEHEAD et al. (1998) reported that vitamin E can alleviate the depression in egg production in heat stressed laying hens. During heat stress, hepatic synthesis of vitellogenine, a protein precursor for yolk formation, and its release into the blood were impaired (BOLLINGIER-LEE et al., 1998; WHITEHEAD et al., 1998) leading to decreases of plasma vitellogenine concentrations and of plasma/liver protein ratio. Dietary supplementation with vitamin E improves egg production by facilitating the release of vitellogenine from the liver and by increasing its concentration in the blood (BOLLINGIER-LEE et al., 1998).

Conclusion

Based on the results in our study, it is concluded that dietary supplementation of laying hens with vitamins C and E singly or in its combined form, can at least in part alleviate heat stress induced oxidative damage. This is evidenced by the increase recorded in the egg quality parameters of the treatment groups when compared to the control.

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SAŽETAK

Proveden je pokus s ciljem da se istraže učinci toplinskog stresa na kakvoću jaja nesilica kojima su u hrani dodani vitamin C i E. Ukupno je 720 nesilica L33 u dobi od 39 tjedana bilo podijeljeno u četiri skupine po 180 nesilica. Kontrolna skupina dobivala je osnovnu hranu, dok je jedna pokusna skupina dobivala osnovnu hranu s dodatkom 150 mg l-askorbinske kiseline/kg (vit. C skupina), druga 150 mg α-dl-tokoferol acetata/kg (vit. E skupina), a treća skupina dobivala je osnovnu hranu s dodatkom 150 mg l-askorbiske kiseline/kg i 150 mg α-dl-tokoferol acetata/kg (vit C+E skupina). Težine bjelanjka ($P<0,05$) i ljuske ($P<0,005$) bile su veće u skupini

koja je dobivala vitamin E i skupini koja je dobivala vitamine C+E u usporedbi sa skupinom koja je dobivala samo vitamin C i kontrolnom skupinom. Žutanjak je bio teži (P<0,05) u skupini koja je dobivala vitamin C+E u usporedbi sa skupinom koja je dobivala vitamin E (P<0,005), vitamin C (P<0,0005) i kontrolnom skupinom. Razlika u debljini ljuske jajeta, čvrstoći jajeta i specifičnoj težini bila je na razini P<0,05 u skupini C+E u usporedbi sa skupinama koje su dobivale C vitamin i E vitamin (P<0,005) i kontrolnom skupinom. Haughova jedinica bila je veća (P<0,05) u skupini koja je dobivala vitamine C+E u odnosu na skupine koje su zasebno dobivale vitamin C ili E (P<0,005) i kontrolnu skupinu. Rezultati pokazuju da dodatak antioksidantnih vitamina ima povoljan učinak na kakvoću jaja u nesilica izloženih toplinskom stresu.

Ključne riječi: toplinski stres, vitamin C, vitamin E, kakvoća jaja, nesilice