

Effects of vitamin E and vitamin C dietary supplementation on egg production and egg quality of laying hens exposed to a chronic heat stress

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SUMMARY

In this study, the effects of vitamin E (α -tocopheryl acetate) and vitamin C (L-ascorbic acid) supplementation in diets were investigated on feed consumption, egg production, egg quality in laying hens exposed to chronic heat stress. The experiment was carried out from July 15th to September 15th. A total of 120 Hyline-White Leghorn hens, 150 days old, were divided into four groups of 30 birds. One group was fed with basal diet (control group) and treatment groups were fed with the basal diet supplemented with either 125 mg of α -tocopheryl acetate /kg of diet (Vit E group), and 200 mg of L-ascorbic acid /kg of diet (Vit C group) or 125 mg of α -tocopheryl acetate /kg of diet plus 200 mg of L-ascorbic acid of diet (Vit E + C group). Although feed intake among groups did not significantly differ, mortality has significantly decreased in supplemented birds compared to the controls ($p < 0.05$), particularly in the Vit E + C group. In the same way, feed efficiency and body weight gain were significantly improved in birds co-supplemented by the 2 vitamins ($p < 0.05$). Egg production and egg weight were also significantly increased in treated birds ($p < 0.05$). Slight increases of egg specific gravity and shell thickness were observed in supplemented birds, particularly in Vit E + C group, whereas percentages of egg albumen, of egg shell and haugh units were not modified. Moreover, the percentage of egg yolk was markedly enhanced in treated animals ($p < 0.05$): this parameter was maximal in Vit E + C group, followed by Vit E group, then Vit C group and controls.

These results show that these 2 anti-oxidant compounds, vitamin E and vitamin C, have some protective actions against heat stress-induced-deleterious effects. Therefore, dietary supplementation by 125 mg vitamin E plus 200 mg vitamin C/kg of diet may increase egg production and improve egg quality in laying hens during heat stress.

Keywords : Heat stress - vitamin E - vitamin C - laying hen - dietary supplementation - egg production.

RÉSUMÉ

Effets d'une supplémentation alimentaire en vitamine E et en vitamine C sur la production et la qualité des œufs chez des poules pondeuses exposées à un stress thermique chronique. Par M. ÇİFTÇİ, O. NIHAT et T. GÜLLER.

Dans cette étude, les effets possibles d'une supplémentation alimentaire en vitamine E (acétate d' α -tocophérol) et/ou en vitamine C (acide ascorbique) sur la consommation alimentaire, la production et la qualité des œufs ont été recherchés chez des poules pondeuses exposées à un stress thermique. L'expérience a été réalisée du 15 juin au 15 Septembre sur un total de 120 poules White Leghorn âgées initialement de 150 jours réparties en 4 groupes de 30 animaux. Un groupe (groupe témoin) n'a reçu que l'aliment de base, les 3 autres groupes ont été supplémentés par la vitamine C seule (200 mg / kg d'aliment) (groupe Vit C), par la vitamine E seule (125 mg / kg d'aliment) (groupe Vit E) et par les 2 vitamines à la fois (200 mg de vitamine C et 125 mg de vitamine E / kg d'aliment) (groupe Vit C + Vit E).

Alors que la consommation d'aliment n'a pas été significativement modifiée d'un groupe à l'autre, une diminution significative de la mortalité a été observée chez les oiseaux supplémentés ($p < 0.05$), particulièrement dans le groupe Vit C + Vit E. De même, l'efficacité de la ration et le gain de poids ont été nettement améliorés dans ce groupe ($p < 0.05$). La production d'œufs et leur poids ont également été plus élevés chez les animaux traités que chez les témoins ($p < 0.05$). De faibles augmentations de la densité des œufs et de l'épaisseur de la coquille ont été aussi obtenues chez les animaux supplémentés alors que les pourcentages d'albumine, de la coquille et les unités haugh n'ont pas été modifiés. Mais surtout, le pourcentage de jaune a été notablement accru chez les oiseaux co-supplémentés en vitamines E et C.

Ces résultats montrent que les vitamines anti-oxydantes E et C permettent de prévenir au moins partiellement les effets délétères d'un stress thermique chronique et qu'une co-supplémentation alimentaire des poules pondeuses par 125 mg de vitamine E et 200 mg de vitamine C augmente la production en œufs et en améliore la qualité.

Mots-clés : Choc thermique - vitamine C - vitamine E - supplémentation alimentaire - production en œufs.

Introduction

Heat stress in laying hens is prompted by combinations of environmental temperature and humidity that prevent the bird's thermoregulatory process from effectively dissipating the heat produced during metabolism [32]. High environmental temperature is the major problem faced by laying hens as well as poultry farmers usually in summer months. The ideal temperature for laying hens is about 20°C [21]. Heat stress begins when the ambient temperature climbs above 25°C and is readily apparent above 30°C. Heat stress in laying hens reduces live weight gain, feed intake, feed efficiency, production and quality of eggs and increases mortality [4, 7].

The researchers have tried to minimize the effect of heat stress by changing the environment and diets of laying hens.

Environmental approaches include increasing the airflow over birds to increase heat loss, increasing ventilation rates, or using evaporative cooling systems in enclosed houses and lowering stocking densities. Nutritional modifications usually made are the optimization of diets for covering the altered needs of stressed birds for protein and energy and for providing some additional nutrients. Because it is expensive to cool poultry houses, methods are focused mainly on nutritional modifications. For this aim, vitamin C and vitamin E are used in the poultry diet because of their anti-stress effects and also because their synthesis are reduced during heat stress [3, 9, 11, 14, 20]. Vitamin E serves as a physiological anti-oxidant through inactivation of free radicals. BOLLEN-GIER-LEE *et al.* [3] reported that heat stress impairs the synthesis and release of vitellogenine and that dietary supplementation with Vit-E facilitates release of vitellogenine

necessary for yolk formation. In the same way, under hot conditions, birds are not able to synthesize sufficient amounts of ascorbic acid [15] and supplemental ascorbic acid could significantly reduce the body temperature [23, 25, 29]. Therefore, mortality rate observed during heat stress with adequate ascorbic acid supply is generally lower [14]. DAVID and BRAKE [6] found that 1000 ppm ascorbic acid supplementation in broilers reduced mortality by 14.6%. During heat stress, corticosterone increases conversion of nor-epinephrine to epinephrine, which induces degeneration of ovarian follicles [18].

The objective of this study was to determine the possible beneficial effects of dietary vitamin E and vitamin C supplementation on feed consumption, and particularly on egg production and qualities (weight, mortality, shell thickness) in laying hens exposed to a chronic heat stress.

Material and methods

ANIMALS

A total of 120 Hyline White Leghorn, 150-day-old, hens were divided into four groups of 30 hens. Each group contained 5 hens in 6 cages. Hen house was provided with 17 h light per day. The hens were randomly assigned according to initial body weights. Feed and water were given *ad libitum*. The hens were vaccinated against Marek and Newcastle diseases. Similar management conditions were maintained for all groups. Temperatures and humidity were recorded at a particular time daily (08.00, 14.00, 20.00 and 02.00 h within the experimental house). The experiment was carried out from July 15th to September 15th.

DIETARY TREATMENTS

Treatment groups were fed with basal diet (control group) or basal diet supplemented with either 125 mg of α -tocopheryl acetate /kg of diet (Vit E group), 200 mg of L-ascorbic acid /kg of diet (Vit C group) or 125 mg of α -tocopheryl acetate /kg of diet plus 200 mg of L-ascorbic acid / kg of diet (Vit E + C group). Vitamin C (ROVIMIX® STAY-C® 35) and vitamin E (ROVIMIX® E-50 SD) were provided by a commercial company (Roche, Levent-Istanbul, Turkey). Ingredients and chemical composition of the basal diet are shown in Table I. The basal diet was a typical layer diet containing 2950 kcal/kg metabolic energy (ME) and 17.50 % crude protein (CP), and was calculated to meet or slightly exceed the nutrient requirements recommended by the National Research Council [22].

SAMPLE COLLECTION AND LABORATORY ANALYSIS

Feed consumption and egg production were recorded daily. Egg weight, body weight and egg specific gravity were measured every two weeks. Specific gravity was individually measured with saline solutions with specific gravities ranging from 1.055 to 1.095 by 0.005 units. Haugh units were measured on two eggs per cage [8]. Furthermore, two eggs per cage were collected to determine the proportions of

Ingredients	Diet. %
Corn Grain	67.20
Soybean Meal	15.50
Wheat Grain	4.30
Sunflower Meal	0.30
Fish meal	3.00
Limestone	8.00
Dicalcium Phosphate	1.00
Vitamin Premix ¹	0.25
Mineral Premix ²	0.10
NaCl	0.25
Antioxidant	0.10
Chemical Analyses (DM basis)	
Dry Matter, %	90.31
Metabolizable Energy, kcal/kg	2750
Crude Protein, %	17.50
Calcium, %	3.42
Phosphorus, %	0.60

TABLE I.—Ingredients and chemical composition of the basal diet of laying hens.

¹ Per kilogram vitamin premix provided the following (Rovimix 124/V); 7.500.000 IU vitamin A; 1.500 IU cholecalciferol; 7.500 IU vitamin E; 1250 mg; menadione; 500 mg thiamin; 5000 mg riboflavin; 1000 mg folic acid; 50 mg; biotin. Per kilogram vitamin premix provided the following (Reminal: 40.000 mg Mn; 12.500 mg Fe; 25.000 mg Zn; 3.500 mg Cu; 150 mg Iodin; 75 mg Se; 175.000 mg Cholin Chloride.

albumen, yolk, shell and yolk solid. The shell thickness of individual eggshells was measured with a micrometer on three pieces of membrane-free shell taken from the equator of an egg. During the treatment, mortality in each group was recorded. Chemical analyses of the diet and feces samples were run using international procedures of AOAC [1].

STATISTICAL ANALYSES

All data were analysed by analysis of variance procedures (ANOVA) and Duncan multiple-range test [28]. Results were considered as significant when P values were less than 0.05.

Results

During the experiment, hen houses temperature and humidity were represented in Figure 1. While the observed house temperatures were between 23 and 35°C, maximum and minimum values of house relative humidity were obtained between 45% and 78%.

Table II shows the results of dietary Vit E, Vit C and Vit E+C supplementation on zootechnical performance parameters. Feed consumption was not affected by vitamin E, vitamin C and vitamin E+C supplementation (as compared to control groups (P>0.05)). Mortality rate was the highest in control group than gradually decreased in Vit E, Vit C and Vit E+C groups (P<0.05). Final body weight, feed efficiency and hen-day egg production were significantly greater in Vit E + Vit C group (P<0.05) compared to control or Vit E

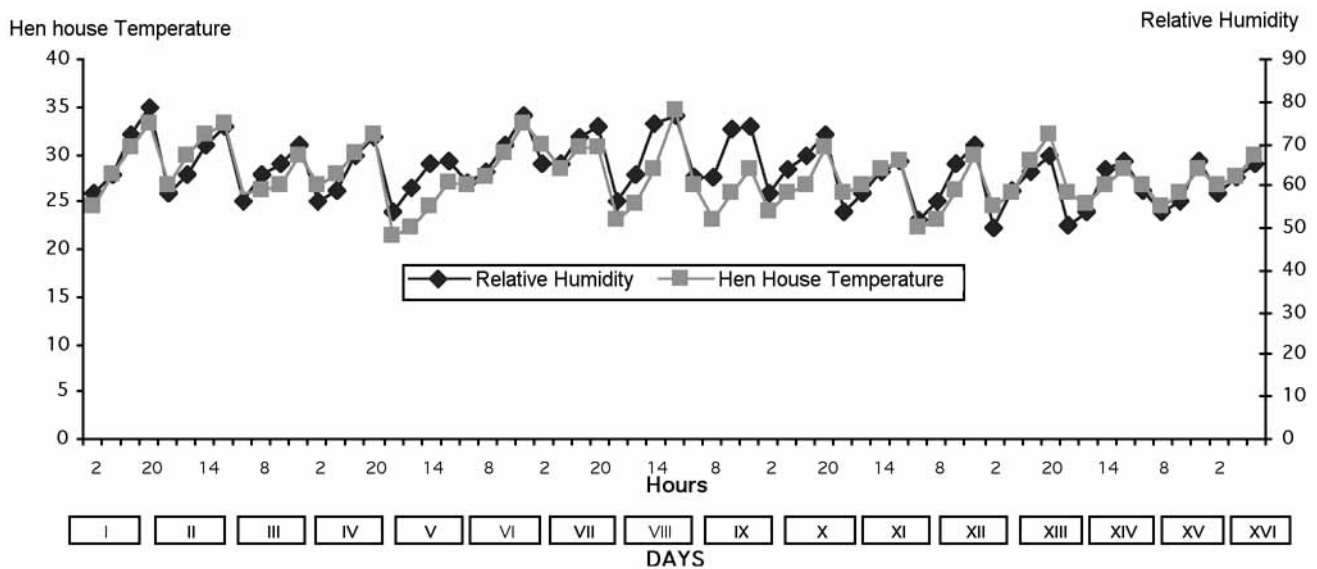


FIGURE 1.—Variations of hen house temperature (°C) and relative humidity (%) observed during the experimentation.

Parameters	Treatments				P
	Control	Vit. E	Vit. C	Vit E+C	
Feed Intake (g/hen/day)	92.02 ± 3.55	93.08 ± 2.60	93.09 ± 2.25	94.07 ± 2.78	NS
Body weight initial (g)	1459.2 ± 4.89	1451.3 ± 4.26	1450.1 ± 4.85	1445.7 ± 5.81	NS
Body weight final (g)	1650.5 ^a ± 2.55	1680.0 ^a ± 2.21	1685.5 ^{ab} ± 3.55	1697.0 ^b ± 4.31	<0.05
Mortality (%)	6.25 ^a ± 0.35	5.08 ^b ± 0.26	4.06 ^c ± 0.21	3.01 ^d ± 0.15	<0.05
Feed efficiency (g feed/g egg)	2.21 ^a ± 0.10	1.88 ^a ± 0.08	1.85 ^{ab} ± 0.08	1.72 ^b ± 0.05	<0.05
Hen-day egg production, (%)	82.25 ^d ± 0.80	84.25 ^e ± 0.72	85.92 ^b ± 0.83	88.29 ^a ± 0.70	<0.05
Egg Weight (g)	58.7 ^a ± 0.36	59.8 ^b ± 0.31	59.6 ^b ± 0.41	59.8 ^b ± 0.29	<0.05

TABLE II.—Effects of dietary supplementation with vitamin E and C on growth performances and egg production in laying hens reared during heat stress. Results are expressed as means ± standard deviations.

^{a-d} : Mean values within a row with no common superscript differ significantly ($P < 0.05$).

Item	Treatments				P
	Control	Vit. E	Vit. C	Vit E+C	
Egg albumen (%)	64.22 ± 5.3	64.98 ± 4.6	64.25 ± 3.87	65.51 ± 5.21	NS
Egg yolk (%)	22.01 ^d ± 0.52	25.09 ^b ± 0.50	24.01 ^c ± 0.56	26.55 ^a ± 0.65	<0.05
Egg specific gravity (g/cm ³)	1087.1 ± 1.85	1089.4 ± 1.7	1088.6 ± 1.82	1090.2 ± 1.90	NS
Haugh unit	88.50 ± 2.21	87.62 ± 2.28	89.21 ± 2.36	88.21 ± 2.24	NS
Eggshell thickness (mm)	0.358 ± 0.06	0.367 ± 0.05	0.362 ± 0.05	0.375 ± 0.08	NS
Eggshell (%)	12.10 ± 0.30	12.25 ± 0.35	12.35 ± 0.21	12.51 ± 0.20	NS

TABLE III.—Effects of dietary supplementation vitamin E and C on egg components and quality. Results are expressed as means ± standard deviations.

^{a-d} : Mean values within a row with no common superscript differ significantly ($P < 0.05$).

groups. Moreover, egg weight was significantly increased in all treated groups ($P < 0.05$).

Table III shows egg components and quality factors. Vitamin E or vitamin C supplementation affected nor the percentage of albumen, and of eggshell and neither haugh units ($P > 0.05$). However, the egg specific gravity and shell

thickness appeared to be slightly increased in Vit E and in Vit C groups in comparison to control birds, although differences were not significant ($P > 0.05$). But, the highest values of these parameters were observed in Vit E + Vit C group. Moreover, the percentage of egg yolk was significantly greater ($P < 0.05$) in Vit E and in Vit E + Vit C groups than in control and in Vit C groups.

Discussion

In our study, the simultaneous dietary supplementation with vitamin E and vitamin C of laying hens exposed to heat stress have significantly improved zootechnical performances (increases of feed efficiency and of body weight gain, decrease of mortality) and egg qualities (production, weight, specific gravity, shell thickness and the percentage of yolk). Additions of vitamin E or vitamin C alone into diets appeared to be less beneficial for laying hens during heat stress.

Vitamin E has been demonstrated to be an antioxidant that scavenges the free radicals generated in cell membranes [2, 35]. In addition, vitamin C itself plays important roles in cellular anti-oxidant defenses, not only by reacting with all oxygen species through formation of dehydroascorbyl, a particular inert radical, but also by transferring radical equivalents from lipid phases to aqueous compartment [13]. In complement, ascorbate participates to the regeneration of reduced glutathione from oxidized form in the cytoplasm and allows tocopherol regeneration through a non-enzymatic reaction [16]. The synergic effects between these two vitamins are particularly efficient for reducing production of reactive oxygen species. Heat stress leads to generation of free radicals, such as O_2^- and HO. These free radicals can damage cell membranes by inducing lipid peroxidation of polyunsaturated fatty acids in the cell membrane [22]. Because radical reactions are exergonic, they contribute with failure of thermoregulation process to the increase of body temperature observed during heat stress. Consequently, dietary supplementation of birds with vitamin E, vitamin C or a combination of these 2 anti-oxidant compounds would 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. These beneficial protective effects of vitamins were evidenced by increases of body weight gain and of egg production and qualities in supplemented laying hens in comparison to control birds. In the same way, previous reports have shown that elevated dietary vitamin E can enhance disease resistance in pullets raised under heat stress conditions of 34°C for 14 h/day and 24°C for 10 h/day [31], or that supplemental ascorbic acid was able to significantly reduced the body temperature [5, 10, 23]. Besides, SCHEIDLER and FRONING [27] have demonstrated that egg production was significantly improved in hens fed with various flaxseed diets with high level of vitamin E (50 IU/kg diet) compared to hens fed with the same diets containing low level of vitamin E (27 IU/kg diet). During heat stress, hepatic synthesis of vitellogenine, a protein precursor for yolk formation, and its release into blood were impaired [3, 33] 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 into blood [3]. In recent experiments conducted by WHITEHEAD *et al.* [33], layers were maintained in controlled environment housing at 22°C, then held for 1 month at

32°C, and finally returned to 22°C. Dietary Vitamin E levels of 315 IU/kg resulted in higher rates of lay and better feed conversion efficiency during the hot period and in the following months, suggesting that the NRC [22] recommendations of 5 IU/kg for laying hens is too low for birds held in hot climates. In addition, the possible benefits of higher vitamin E supplementation on performances of birds subjected to long-term exposure to high temperatures (tropical heat conditions) have to be explored. Because heat stress increases the needs in anti-oxidant vitamins and because birds cannot synthesize enough ascorbate during hot conditions [5, 10, 23], dietary supplementation with high dosages of vitamins E and/or C would be conducted. In our study, we observed only slight positive effects of supplementation with ascorbate alone on bird performances (growth and egg production). The weakness of these effects would be resulted from an insufficient dosage of vitamin C, unable to totally recover ascorbate requirement under hot conditions. NIJOKU and NWAZOTA [19] demonstrated that high dietary vitamin C (200, 400, 600 mg/kg) supplementation significantly increased egg production in hens exposed to heat stress. Similarly, DEMIR *et al.* [7] reported that vitamin C supplementation in feed (200 mg/kg) during heat stress increased feed intake and eggshell thickness.

On the other hand, bioavailability of nutrients is affected by heat stress : ZUPRIZAL *et al.* [35] have shown that true digestibility of proteins and amino acids from two different protein sources (rapeseed and soybean meals) decreased as the temperature increased from 21 to 32°C [34]. Activities of trypsin, chymotrypsin, and amylase significantly decreased at high temperature (32°C) [12]. The reason for the decrease in activity of these digestive enzymes is uncertain. But it is probable that the enzyme optimal temperatures were below 32°C. Several studies [24, 26, 29, 30] have demonstrated that dietary supplementation with vitamin E and/or vitamin C alleviates the heat stress negative effects on apparent nutrient digestibility. Mc KEE and HARRISON [17] also detected an improvement in feed conversion ratio of broilers as a result of vitamin C supplementation during heat stress. It is well known that vitamin C improves iron assimilation by reduction of Fe^{3+} into Fe^{2+} , which is more assimilated by intestine and thereby vitamin C improves resistance to infections. Locally, oxidative lesions leading to conformational modifications of proteins could induce pancreatic enzyme inhibition and/or dietary protein resistance to digestion. Consequently, the presence of anti-oxidants (vitamin E and/or C) could partially interfere with oxidative protein denaturation and would improve digestibility of nutrients and feed efficiency.

In conclusion, dietary supplementation of laying hens with anti-oxidant vitamins (vitamin E or vitamin C or a combination of the both compounds) can attenuate heat stress induced oxidative damage. These positive effects were evidenced by increases of growth performances, egg production and improvement of egg qualities in comparison to non-supplemented birds. Moreover, supplementation with both vitamin E and vitamin C is the most efficient treatment probably because these two anti-oxidant compounds act in synergy during reactive oxygen species inactivation. Nevertheless,

further investigations will be performed for evaluating the potential beneficial effects of anti-oxidant vitamins during long-term exposure to heat stress and for characterizing more precisely the vitamin actions during yolk formation.

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