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Vitamins C and E can Alleviate Adverse Effects of Heat Stress on Live Weight and Some Egg Quality Profiles of Layer Hens

J. J. Ajakaiye[1]*, M. Cuesta-Mazorra and J. R. Garcia-Diaz

Department of Zootechnics and Veterinary Medicine, Faculty of Agricultural Sciences, Universidad Central "Marta Abreu" de Las Villas, Km 5½ Carretera a Camajuani, P. O. 54830, Santa Clara, Villa Clara, Cuba; [1]College of Agriculture and Animal Sciences, Ahmadu Bello University, Mando Road Campus, Kaduna, Nigeria
*Corresponding author: joachim@uclv.edu.cu

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ABSTRACT

An experiment was designed to investigate the effects of vitamins C and E on live weight and egg quality profile of layer hens subjected *in situ* to heat stress. Hybrid White Leghorn (L_{33}) layer hens (n=720) and 39 weeks old, were randomly allotted to 4 treatments containing 0, 150 mg vitamin C, 150 mg (150 IU) vitamin E, and 150 mg vitamin C plus 150 mg vitamin E/Kg of diet. Final live weight in supplemented groups were significantly (P<0.05) higher than control. Egg, egg yolk and egg albumen weight in groups administered with vitamins E and C+E were very significantly higher compared with control, meanwhile, the same indicators were only significantly (P<0.05) higher in vitamin C group compared with control. Similarly, eggshell weight was significantly (P<0.05) higher in all treated groups, with the highest value recorded in group supplemented with vitamins C+E compared with control. By sustaining bird's live weight and increasing egg quality indicators, it is concluded that both antioxidants offered protective effect against the thermally stressful hot-humid condition.

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INTRODUCTION

In recent times, poultry production has occupied the center stage due to its comparative advantage in cost of production relative to other species such as cattle and piggery; advancement in genetic selection, high biological and nutritive values; and the general acceptability in consumption of its products, that is, meat and eggs. Unfortunately, achievement in this sector is been hampered by today's rising global warming, because birds like all mammals are homoeothermic; they are able to maintain their body temperature within certain limits. At homeothermy, oxidants are formed in birds as a normal product of aerobic metabolism, but at high ambient temperature (AT) and relative humidity (RH) exposure, bird's heat load is increased due to the environmental heat gain and the energy cost associated with activation of metabolic processes required for heat dissipation. Therefore, various researchers have suggested the use of antioxidants vitamins C and E, especially in its combined form as an effective strategy at alleviating the negative effects of heat stress in poultry (German and Traber, 2001; Bolukbasi *et al.,* 2007).

Although birds are renal synthesizer of vitamin C, it is well documented that usage far outweighs production under praxis condition (Maurice *et al.,* 2002). Vitamin E on the other hand, is not synthesized by birds, and its availability is through exogenous administration only (NRC, 1994). Furthermore, several works have been reported on the palliative roles of these antioxidants in chickens and Japanese quails either in its single or combined form, but under controlled or research conditions (Ciftci *et al.,* 2005; Khan and Sardar, 2005; Bolukbasi *et al.,* 2007), however, information are lacking on the use of these vitamins *in situ*, under variable climatic conditions.

The objective of the present study, therefore, were to determine the effects of vitamins C and E supplementation on live weight and some egg quality profiles of White Leghorn (L_{33}) layer hens, reared under hot-humid condition during the summer period.

MATERIALS AND METHODS

Experimental site

The study was conducted at the poultry production unit of "Las Casas II", situated in the province of Villa

Clara; it is located between 22° 53′ N and 82° 02′ W, with an altitude between 90-100 meters above sea level. Total precipitation during the study period was 327.2 mm, while average air velocity was 3.15 m/s. It experiences a typical sub-tropical maritime climate, with an annual average air temperature and relative humidity of 35.9 °C and 75% respectively, especially in the months of July and August. Meteorological data consisted of ambient temperature (AT) and relative humidity (RH) were measured daily with standard instruments throughout the study period.

Experimental birds

Commercial White Leghorn (L_{33}) layer chickens (n=720), 39 weeks old and an average live weight of 1.8 ± 0.04 Kg were used in the experiment. The birds were randomly divided *in situ* within production pen into four groups of 180 each, and each group into four replicates of 45 birds, and three birds/cage of 0.4×0.4 m dimension, at a stocking rate of 500 cm^2/bird. One group was fed with basal diet (control group) and treatment groups were fed with the basal diet supplemented with 150 mg vitamin C(l-ascorbic acid), 150 mg (150 IU) vitamin E (α-dl-tocopherol acetate), and 150 mg vitamin C plus 150 mg vitamin E/Kg of diet. Prior to the experiment the birds were duly dewormed and vaccinated. The birds were fed recommended NRC (1994) basal diet of 110 g/bird/day, and water was given *ad libitum.* Feed constituents and bromatological analysis of basal diet calculated according to AOAC (1990) are shown in Table 1.

Measurement of live weight and egg quality parameters

Live weight of 20 birds were randomly selected within replicates of each group and measurements were recorded with a standard electronic weighing balance (Salter, Pocket Balance, England) with a maximum calibration of 5Kg and a precision of 0.1 g. Egg yolk and albumen weight measurement was carried out on individual 10 eggs that were randomly selected within replicates of each group, and measurement was conducted twice a week and for four weeks (Amaefule *et al.*, 2004). Eggshell weight and Egg weight measurements were carried out as described by Roberts (2004).

Statistical Analysis

The PC STATISTICA 8.0 package was used. Data for ambient temperature and relative humidity; and live weight were analyzed using Student's paired *t* test, and expressed as mean standard deviation and standard error of the mean (Mean±SEM) respectively. P value less than 0.05 were considered significant. Data on egg quality parameters were analyzed using the General Linear Models procedure for ANOVA (SAS Institute, 1994). The statistical analysis of data involved determination of arithmetic means ($\bar{x}$) and standard error of the mean (Mean±SEM). Significant differences among treatment means were identified using Duncan's multiple-range test (Duncan, 1955) and P<0.05 were considered significant.

Table 1: Composition and calculated bromatological analysis of basal diet fed to White Leghorn (L_{33}) layer hens during the hot-humid period.

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	
ME, 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

[(a)] Vitamin supplement per (kg) of diet: Vitamin A, 12000 IU; vitamin D_3, 2500 IU; vitamin E, 5 IU; 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. ME = Metabolizable energy; CP = Crude protein; P(a) = Available phosphorus.

RESULTS

Throughout the study period, the AT outside and inside the pen showed a similar pattern of increase from 9:00 a.m. to 3:00 p.m., and subsequently decreased between 3:00 p.m. to 6:00 p.m. Outside AT was higher (P<0.05) than inside (Table 2) . However, the RH mean value of 84.6% inside the pen was significantly (P<0.05) higher than 81.5% obtained outside the experimental pen.

Final live weight in supplemented groups was significantly (P<0.05) higher than control (Table 3). Egg, egg yolk and egg albumen weights in groups administered with vitamins E and C+E were very significantly higher compared with control, meanwhile, the same indicators were only significantly (P<0.05) higher in vitamin C group compared with control. Similarly, eggshell weight was significantly (P<0.05) higher in all treated groups, with the highest value recorded in group supplemented with vitamins C+E compared with control (Table 4).

DISCUSSION

The mean AT of 31.3 and 33.0 °C observed inside and outside the experimental pen during the study period were outside the thermoneutral zone of 18-22 °C established for this specie (Holik, 2009). This high AT in

Table 2: Meteorological data of ambient temperature and relative humidity at the experimental site during the study period.

Hour	Ambient Temperature (^oC)			Relative Humidity (%)		
	Out	In	± SD	Out	In	± SD
9:00 a.m.	31.8	29.4	0.5	85.6	88.6	1.7
12:00 noon	35.6	33.3	0.4	75.4	81.4	1.9
3:00 p.m.	35.8	34.0	0.9	78.6	79.6	2.5
6:00 p.m.	29.0	28.5	0.6	86.3	88.9	2.0
Mean	33.0^a	31.3^b	0.6	81.5^b	84.6^a	1.3

Out = Outside the pen, In = Inside the pen, ± SD = Standard deviation. For each parameter, mean values with different superscript alphabets along the same row are significantly (P<0.05) different.

Table 3: Live weight of White Leghorn (L_{33}) layer hens supplemented with vitamins C and E, and reared under hot-humid condition (n=320).

Parameter	Treatment in groups				± SEM
	Vit-C	Vit-E	Vit-C+E	Control	
Initial live weight (g)	1503	1512	1516	1520	35.6
Final live weight (g)	1470^a	1482^a	1490^a	1440^b	34.1

g = Grams; SEM = Standard error of mean. Mean values with different superscript alphabets along the same row are significantly (P<0.05) different.

Table 4: Egg absolute weight and its principal components of White Leghorn (L_{33}) layer hens supplemented with vitamins C and E during the experimental period (n=160).

Parameters/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	0.19
II	60.78^a	61.93^a	61.99^a	57.60^b	0.31
III	60.77^b	62.09^b	63.69^a	56.32^c	0.28
IV	61.17^b	64.97^a	64.98^a	56.96^c	0.37
Egg yolk weight (g)					
I	15.64^{ab}	15.35^{bc}	15.86^a	15.16^c	0.07
II	15.40^{ab}	15.97^a	15.97^a	15.11^b	0.10
III	15.29^b	15.54^b	16.24^a	14.66^c	0.10
IV	15.83^b	16.59^a	16.59^a	14.76^c	0.11
Egg albumen weight (g)					
I	39.81^b	41.36^a	40.60^a	38.17^c	0.14
II	39.58^a	40.02^a	40.38^a	37.23^b	0.25
III	39.75^b	40.98^{ab}	41.45^{ab}	36.18^c	0.18
IV	39.60^b	42.40^a	42.12^a	36.90^c	0.27
Eggshell weight (g)					
I	4.94^b	5.25^a	5.30^a	5.16^{ab}	0.05
II	5.82^a	5.95^a	5.65^a	5.27^b	0.06
III	5.73^b	5.97^a	6.00^a	5.48^c	0.04
IV	5.75^b	5.99^b	6.27^a	5.30^c	0.05

SEM = Standard error of the mean; g = Grams. Means with different superscripts alphabets along the same row are significantly (P<0.05) different.

combination with above 80% RH recorded throughout the experimental period showed that the birds were exposed to a thermally stressful condition, as evidenced by heavy panting, darkened skin color due to blood being brought to the skin and behavioral manifestations. All experimental birds, and particularly those in control groups, were observed to lift their wings away from body, constantly drank water and wet their crests and wattles; some were observed to stretch the ventral portion of their neck longitudinally along the lower side of the wire netting of the cage especially in regions directly under the water in an attempt to dissipate heat through conduction. Birds were constantly seeing to be drinking, but ate less feed. Similar observations were made by Gutierrez *et al.* (2009) after a 30 day experiment, involving seventy two 123-day-old Hy-line brown layers subjected to a controlled 30 °C AT and supplemented with chilled drinking water. The authors recorded lower panting rate, higher feed intake and conversion in supplemented birds compared to control. At this temperature range the body oxygen uptake is known to increase many fold. This causes an O_2 flux which leads to the conversion of some O_2 into intermediate products referred to as reactive oxygen species (ROS) known to be toxic to the body (Alok *et al.*, 2003).

The increase in live weight observed in this study in supplemented groups relative to control were confirmed by Konka *et al.* (2009) and Ajakaiye *et al.* (2010) who reported increased feed intake and liveweight gain in broilers and transported layer hens supplemented with vitamin C and or E, during the hot summer period respectively. The observed reduction in live weight particularly in control group could probably be due to the combination of high AT and RH which aggravated the intensity of cell damage in the birds. Furthermore, authors such as De Basilio and Picard, (2002); Mashaly *et al.* (2004) and Pavlik *et al.* (2009) reported that reduced feed intake was the major cause of poor live weight, and reduced egg morphometric profiles, because enhanced enzymatic and non-enzymatic antioxidants systems were duly overwhelmed at both high AT and HR.

Similarly, Khan and Sardar (2005) reported an increase in egg production, egg weight and eggshell thickness in Desi, Fayoumi and commercial White Leghorn layer chickens exposed to an 8 week hot-summer (June and July) period and supplemented with vitamin C. However, in a separate experiment, the report of Yardibi and Turkay (2006), who observed a reduction on metabolic consequences of heat stress, but no effect on egg quality, after subjecting 150 Leghorn laying hens to a 9-week study at a constant AT of 35 °C and RH of 65%, and supplemented with 30, 80 and 105 mg of vitamin E/kg are not in agreement with the result of this study. The results obtained in their experiment may probably be due to iatrogenic effect, low dosification, or hereditary characteristics (Melesse *et al.,* 2010), and non-variability of climatic condition to which the birds were subjected. As Eid *et al.* (2008) reported that egg yolk LPO associated with oxidative stress, egg weight and egg production were improved in layer hens subjected to a 7-day oxidative stress induction, through dexamethasone administration and supplemented with 200 mg of vitamin E/kg diet, while Bolukbasi *et al.* (2007) reported a significant ($P<0.05$) increase in egg yield in 128 Lohmann LSL hybrid layer hens supplemented with 45, 65 and 85 UI/kg of vitamin E and subjected to a 42-day exposition at 30 °C. These authors concluded that egg production was significantly greater ($P<0.01$) with 85 IU /kg vitamin E (81%) as compared to control hens. On the other hand the average egg yields were significantly decreased (approximately 14%) by the heat stress, while concentration of vitamin E in the yolk and plasma linearly increased as the dietary vitamin E increased. Furthermore, it is well documented that nutritional strategies should be aimed at feeding birds towards evening or the early hours of the day under hot-variable conditions, owing to the common knowledge that these periods of the day are relatively fresh (close to or similar to thermoneutral zone) and birds tends to eat more (Wiernusz, 1998; Daghir, 2009).

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 live weight and egg quality profiles of: egg weight, egg yolk weight, egg albumen weight and eggshell weight. However, it is interesting to note that supplementation of both antioxidants appeared to be more beneficial for laying hens during heat stress. Probably, due to their synergistic actions in quenching FRs generated during heat stress (Ciftci *et al.,* 2005). 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, and because radical reactions are exergonic, they contribute to the failure of thermoregulatory process in hyperthermia observed during heat stress (Mujahid *et al.,* 2005).

Conclusion

By increasing live weight and egg quality profiles, the administration of both antioxidants at a dose of 150 mg/kg diet each, demonstrated their positive effects at ameliorating the adverse conditions of the hot-humid summer period under which the birds were reared. It is therefore recommended that both antioxidants be incorporated into laying hen's daily routine feeds as a nutritional strategy towards sustaining live weight and egg quality productions, particularly during the hot summer periods.

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