Revista Brasileira de Ciência Avícola
ISSN: 1516-635X
revista@facta.org.br
Fundação APINCO de Ciência e Tecnologia Avícolas
Brasil

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Revista Brasileira de Ciência Avícola, vol. 12, núm. 3, julio-septiembre, 2010, pp. 205-209
Fundação APINCO de Ciência e Tecnologia Avícolas
Campinas, SP, Brasil

Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Jul - Sep 2010 / v.12 / n.3 / 209 - 209

Technical Note

Effects of Vitamin C and E Dietary Supplementation on Erythrocyte Parameters of Layer Chickens Reared in High Ambient Temperature and Humidity

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■ **Keywords**

Erythrocyte index, heat stress, hematocrit, hemoglobin.

Arrived: November/2009
Approved: April/2010

ABSTRACT

A 30-day experiment involving 720 White Leghorn (L33) layer chickens of 39 weeks of age and average live weight of 1.8 ± 0.04 kg was carried out in a completely randomized design to evaluate the effects of vitamins C and E on erythrocyte parameters of layers during natural summer conditions. Birds were allotted to 4 treatments containing 0, 150 mg vitamin C, 150 mg vitamin E, and 150 mg vitamin C plus 150 mg vitamin E/kg feed. Each treatment was replicated four times. The exposure of layers to 31±3 °C and 33±0 °C ambient temperature and 84.6% and 81.5% relative humidity, inside and outside the experimental pen, during the study period caused an increase in temperature humidity index 15.5 above the threshold value of 70 established for this species. Total erythrocytes counts were not affected by treatment. However, hematocrit and hemoglobin were significantly ($p<0.001$) different in vitamin-C and E treated groups, individually or in combination, as compared to the control birds. Also, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration of the birds fed vitamins C or E, or vitamins C+E were significantly ($p<0.001$) higher than those in control group. The results of the present study showed that the dietary supplementation of vitamin C and E, particularly as a combination, alleviated the counterproductive effects of high ambient temperature and humidity on the birds.

INTRODUCTION

In spite of the genetic improvement of modern livestock, the combination of high ambient temperature (AT) and relative humidity (RH) continues to cause major environmental distress in poultry, impairing their performance. The fluctuation of these environmental parameters, interferes with bird comfort, reduces production efficiency, increases endogenous heat production, and overloads the bird's thermo-regulatory mechanism (Balnave, 2004). Despite the significant accumulated knowledge on the responses of poultry to high ambient temperatures (AT), the intensification or modification of these responses to relative humidity (RH) has received little attention. RH 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 wide range of RH, which can markedly affect the degree of heat stress experienced by the bird (Balnave, 2004). Tao & Xin (2003) were the first to develop a mathematical model to measure the impact of temperature, humidity, and air velocity index (THVI) on heat-stressed chickens. There are many reports in the literature showing the negative influence of heat stress on bird physiology (Vecerek *et al*., 2002; Franco-Jimenez & Beck, 2007; Sinkalu & Ayo, 2008). However, the relationship between AT and RH and hematological erythrocytes parameters, particularly with mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), which are descriptive of red blood cell status, has been seldom studied. Researchers have tried to mitigate the effects of heat stress by changing the environment and diets of laying chickens. Although the environmental approach through modification of housing is the best option to obtain optimal performance, it is nevertheless very expensive, thereby making nutritional strategies more viable alternatives. (Balnave, 2004; Daghir, 2009). Vitamin C and vitamin E are used in the poultry diets for this purpose due to their antioxidant properties, neutralizing the free radicals generated during heat stress (Ramnath *et al*., 2008). Furthermore, plasma concentrations of these vitamins are reduced under practical conditions (Freeman, 1967; McDowell, 1989; Maurice *et al*., 2002). The aim of this study, therefore, was to investigate the possible beneficial effects of the dietary supplementation of vitamins C and E on erythrocyte parameters of layer chickens exposed to chronic heat stress.

MATERIAL AND METHODS

Location: This study was conducted at the poultry production unit of "Las casas II", situated in the province of Villa Clara. It is located at 22°53´ N and 82°02´ W, at an altitude between 90-100 meters above sea level. Total rainfall 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 during the months of July and August.

Experimental birds: A total of 720 commercial White Leghorn (L_{33}) layer chickens, 39 weeks old and an average liveweight of 1.8 ± 0.04 kg were used in the experiment. Birds were randomly divided *in situ* within production pen into four groups of 180 birds each, and each group was further divided into four replicates of 45 birds, and three birds/cage measuring 0.4×0.4 m. One group was fed a basal diet (control group) and the treatment groups were fed the basal diet supplemented with either 150 mg l-ascorbic acid /kg of diet (Vit. C group), 150 mg 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). Vitamin C and vitamin E were from a commercial company (VMD, n.v./s.a, Arendonk, Belgium). Prior to the experiment, birds were duly dewormed and vaccinated according to UECAN (2002) specifications. In addition, specific gravity fecal flotation method, with modified Sheather's solution (David & Lindquist, 1982), was employed to confirm the absence of helminthes in the birds before the beginning of the experiment. Feed was offered at 110 g/bird/day, while water was given *ad libitum*. Feed ingredients and chemical analysis of the basal diet, calculated according to AOAC (1990), are shown in Table 1.

Table 1: Ingredient composition and chemical analysis of the basal diet.

Nutrients/ingredients	Quantity (kg)
Corn	60.7
Soybean meal	26.8
Vegetable oil	1.1
Calcium carbonate	9.17
Monocalcium phosphate	1.12
Monocalcium	0.07
Sodium chloride	0.3
Vitamin[(a)] and Mineral[(b)] Premixes	0.25
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
Threonine,	0.70
Ca, g	3.52
Tryptophan, g	0.23
P(av), g	0.25
Na, g	0.15
Cl, g	0.13

Source: UEB feed factory, Ministry of Agriculture, Villa Clara (2009). (a) Vitamin supplement per kg of diet: Vitamin A, 12000 IU; vitamin D_3, 2500 UI; vitamin E, 5 IU; vitamin K_3, 4.5 mg; thiamin, 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 per kg of diet: Magnesium, 56 mg; iron, 20 mg; copper, 10 mg; zinc, 50 mg; cobalt, 125 mg; iodine, 0.08 mg. P(av) = Available phosphorus.

Collection of blood samples: Blood samples were collected on day 0 of the experiment, at two weeks and at four weeks, coinciding with the end of the experiment. Twenty birds per treatment were randomly selected and bled in the wing veins using sterile gauge-19 needles and syringes. Five ml of blood were collected and gently poured into previously sterilized glass tubes containing heparin as anticoagulant. Each tube was gently mixed by repeated inversion for 2 min. Samples were immediately submitted to the laboratory for analysis.

Analysis of blood samples: Packed Cell Volume (PCV) was determined by microhematocrit method (Schalm *et al*., 1975). Hemoglobin (H_b) concentration was measured spectrophotometrically by cyanomethemoglobin method of Jain (1993) and Feldman *et al*. (2000), using SP6-500 UV spectrophotometer (Pye UNICAM ENGLAND). Total red blood cell counts (RBC) were estimated using hemocytometer (Schalm *et al*., 1975). Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC) and Mean Corpuscular Hemoglobin (MCH) were calculated from Hb, PCV and RBC (Feldman *et al*., 2000).

Statistical Analysis: PC STATISTICA 8.0 software package was used and all data were submitted to one-way ANOVA using the general linear model procedure of the Statistical Analysis System (SAS User's Guide, 1985). The statistical analysis of data involved determination of arithmetic means ($\boldsymbol{x}$) and standard deviations (SD±). The significance of differences between mean values obtained for each level of the experimental factors was determined by Duncan (1955) post-hoc test, with means that differed at $P < 0.05$ were considered as significant.

RESULTS

The meteorological data during the experimental period are presented in Table 2. Throughout the study period, AT outside and inside the pens showed a similar pattern of increase from 9:00 a.m. to 3:00 p.m., and subsequent decrease between 3:00 p.m. to 6:00 p.m. Outside AT was higher ($p < 0.05$) than inside. However, mean RH inside the pen was significantly ($p<0.05$) higher than outside. The chickens were exposed to high AT of 31.3 °C and RH of 84.6% respectively. The mean maximum and minimum THI (Table 3) during the study period were 93.5 and 77.8 with a range of 15.7. The average value of 85.5 recorded for THI was very high.

The effect of dietary supplementation of vitamins C and E on chicken erythrocytes and their indices are shown in (Table 4). PCV, Hb, MCV, MCH and MCHC were significantly increased ($p<0.001$) when birds were supplemented with vitamins C and E individually or in combination as compared to the control group. The highest PCV (29.12%) and H_b (8.74g/100 ml) were recorded in birds supplemented with vitamins C+E, while the lowest values (25.82% and 7.22g/100 ml, respectively) were recorded in the control birs.

Table 3 - Analysis of temperature and humidity index (THI).

Hours	Average	SD	Minimum	Maximum	Range
9:00 a.m.	83.1	±2.510	79.6	91.0	11.4
12:00 p.m.	88.4	±1.767	85.6	93.5	7.9
3:00 p.m.	89.1	±2.915	78.5	93.1	14.6
6:00 p.m.	81.6	±2.367	77.8	87.0	9.2
Mean	85.5	±4.056	77.8	93.5	15.7

SD = Standard deviation.

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 oC (Holik, 2009) established for poultry in tropical regions. The combination of 31.3 °C recorded for AT and 84.6% recorded for RH gave an average temperature humidity index (THI) of 85.5, which is above the THI threshold of 70 established for poultry (Tao & Xin, 2003; Karaman *et al*., 2007). This is a clear indication that the layer chickens were subjected to heat stress. Total RBC counts in this study were not affected. It has been documented that the total RBC counts and Hb should not be interpreted clinically, as they vary almost exactly in parallel with the PCV. Their function is to allow the calculation of MCV, MCH and MCHC, respectively (Kerr, 2002). Hb and PCV values obtained in the present study are consistent with the reports of Iheukwumene & Herbert (2003), who reported values of 6.0-13g/100 ml 29.0-38.0%. However, the MCHC values of 33.0-35.0 pg recorded by those authors are different from our findings, which is probably due to dehydration suffered by the chickens as a result of a disequilibrium in acid-base balance, leading to respiratory alkalosis (Borges

Table 2 - Meteorological data, considering ambient temperature and relative humidity.

Hour	Ambient Temperature (°C)				Relative Humidity (%)			
	Out	In	SD	p-value	Out	In	SD	p-value
9:00 a.m.	31,8 a	29.4 b	± 0.527	0.000000	85.6 b	88.6 a	± 1.729	0.021388
12:00 p.m..	35,6 a	33.3 b	± 0.421	0.000000	75.4 b	81.4 a	± 1.908	0.000071
3:00 p.m.	35,8 a	34.0 b	± 0.848	0.003532	78.6 a	79.6 a	± 2.529	0.436896
6:00 p.m.	29,0 a	28.5 a	± 0.619	0.242058	86.3 a	88.9 a	± 1.972	0.067726
Mean	33,0 a	31.3 b	± 0.583	0.000024	81.5 b	84.6 a	± 1.312	0.000736

Out = Outside the pen, In = Inside the pen, SD = Standard deviation. For each parameter, mean values with different superscripts in the same row line are significantly (p < 0.05) different.

Table 4 - Effects of supplemental vitamin C and E on erythrocyte and its indices of layer chickens reared at high ambient temperature and humidity (n=80).

Treatment Parameters	Treatment in weeks Initial	2	4	Mean
Hematocrit (%)				
Vit-C	29.50 a	29.20 a	28.60 a	29.08 ^{a}
Vit-E	29.40 a	29.20 a	28.60 a	29.07 ^{a}
Vit-C+E	29.60 a	29.00 a	28.80 a	29.12 ^{a}
Control	29.30 a	27.00 b	21.20 b	25.82 b
SD$\bar{x}$	± 0.239	± 0.184***	± 0.131	± 0.311***
Hemoglobin (g/100 ml)				
Vit-C	8.85 a	8.50 b	8.50 b	8.62 ^{a}
Vit-E	8.86 a	8.40 c	8.40 c	8.55 ^{a}
Vit-C+E	8.87 a	8.76 a	8.60 a	8.74 ^{a}
Control	8.87 a	6.80 d	6.00 d	7.22 b
SD$\bar{x}$	± 0.012	± 0.015***	± 0.018***	± 0.083***
Total RBC counts (× 106/mm^3)				
Vit-C	2.82 a	2.78 a	2.75 a	2.78 ^{a}
Vit-E	2.80 a	2.77 a	2.74 a	2.77 ^{a}
Vit-C+E	2.79 a	2.74 a	2.73 a	2.75 ^{a}
Control	2.81 a	2.70 a	2.68 a	2.73 ^{a}
SD$\bar{x}$	± 0.018	± 0.020	± 0.019	± 0.022
MCV (fl)				
Vit-C	104.71 a	105.09 a	104.45 a	104.75 ^{a}
Vit-E	105.43 a	105.72 a	105.14 a	105.43 ^{a}
Vit-C+E	106.19 a	106.44 a	105.58 a	106.07 ^{a}
Control	104.49 a	100.24 b	79.42 b	94.71 b
SD$\bar{x}$	± 1.106	± 0.844*	± 0.930***	± 1.309***
MCH (pg)				
Vit-C	31.48 a	30.69 b	31.03 a	31.07 ^{a}
Vit-E	31.76 a	30.50 b	30.85 a	31.04 ^{a}
Vit-C+E	31.90 a	32.12 a	31.53 a	31.85 ^{a}
Control	31.70 a	25.23 c	22.45 b	26.46 b
SD$\bar{x}$	± 0.237	± 0.225***	± 0.217***	± 0.363***
MCHC (g/100 ml)				
Vit-C	30.25 a	29.19 b	29.79 a	29.74 ^{a}
Vit-E	30.26 a	28.83 b	29.41 a	29.50 ^{a}
Vit-C+E	30.21 a	30.30 a	29.90 a	30.14 ^{a}
Control	30.45 a	25.29 c	28.33 b	28.02 b
SD$\bar{x}$	± 0.265	± 0.177***	± 0.142***	± 0.272***

Means values with different superscripts in the same row are significantly different. Levels of significance: * (p< 0.05), ** (p<0.01), *** (p<0.001). Results are expressed as means ± standard deviations (SD$\bar{x}$). RBC = red blood cells, MCV = Mean corpuscular volume, MCH = Mean corpuscular hemoglobin, MCHC = Mean corpuscular hemoglobin concentration.

et al., 2003). Moreover, under heat stress, birds loose more water (through panting and urine) than they do when they are in the thermal comfort zone. Decreases in body water content (dehydration) and increases in RH result in a reduced ability to dissipate heat via evaporation and/or through increased peripheral blood flow. As a consequence, birds increase water consumption to compensate for this water loss and to increase heat dissipation capacity, as observed throughout the experimental period, particularly in the control group. However, water retention is reduced due to higher electrolyte excretion in urine and feces (Belay & Teeter 1993). The reported values of 7.06-9.37 g/100 ml Hb, 26.56-34.60 % PCV, and 84.27-163.56 fl MCV by Islam *et al*. (2004) in commercial and local chickens reared in the hot Sylhet region of Bangladesh are in agreement with those obtained in the present study. PCV and Hb values of 29.12 ± 0.88 % and 97.2 ± 2.9 g/L, reported by Oladele *et al*. (2001) in pigeons in the hot-dry region of Zaria, Nigeria, are also consistent with our findings. The PCV, Hb, MCV, MCHC and RBC values observed in the present study were slightly lower in the treatment groups, but considerably lower in the control birds, as compared to the values of 30-40%, 9-13 g/100 ml, 127 fl, 29 g/100 ml, and 3.0x1012 /l, respectively, reported by MVM (1986) in chickens. This observation may probably be due to hereditary characteristic of the strain used. Awotuyi (1990) reported that the PCV of local and commercial chickens in Ghana varied between 32.88-33.20 and 31.30-35.60%, respectively, while Nworgu *et al*. (2003) reported a PCV of 28-30.0% for broiler chicks fed cocoa pod husk meal.

In conclusion, the overall antioxidant potential is possibly more efficient and crucial than individual antioxidant nutrients (German & Traber, 2001). The results of the present experiment showed that the combination of 150 mg vitamin C and 150 mg vitamin E provided significantly protected erythrocyte profile and its indices in L33 layer chickens reared under the deleterious effects of high AT and RH.

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