

Effects of dietary supplementation with vitamin C or vitamin E on cardiac lipid peroxidation and growth performance in broilers at risk of developing ascites syndrome

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Objective—To assess effects of high dietary amounts of vitamin C or vitamin E and oxidative stress on the heart and growth performance of broilers maintained at an altitude of 2,200 m above sea level.

Animals—360 chicks (1-day-old broilers).

Procedure—Birds were randomly assigned to 3 groups (120 chicks/group). Each group of birds was fed a specific diet (control group, basal diet containing 12 mg of vitamin E (DL- α -tocopherol acetate)/kg of feed without additional ascorbic acid; vitamin E group, basal diet supplemented with 75 mg of vitamin E/kg of feed; and vitamin C group, basal diet supplemented with 400 mg of ascorbic acid/kg of feed) throughout the entire 7 weeks of the study. Feed consumption and body weight of chicks were recorded on a weekly basis. Nine randomly selected birds from each group were euthanatized each week. Remaining birds were euthanatized at the end of the study. Samples of cardiac tissues were obtained to measure thiobarbituric acid reactive substances (TBARS), an indicator of oxidative stress.

Results—Vitamin E-supplemented diets resulted in better growth performance, lower rates of feed conversion, and lower TBARS content. Vitamin C-supplemented diets resulted in lower feed consumption and lower rates of feed conversion. When used separately, neither of the vitamins had any effect on mortality attributable to ascites syndrome.

Conclusion and Clinical Relevance—It is recommended that diets supplemented with vitamin C, vitamin E, or both be fed to broilers maintained at an altitude of 2,200 m above sea level to improve growth performance. (*Am J Vet Res* 2002;63:673–676)

Ascites syndrome (AS) is a costly metabolic disease of broilers.¹ In Mexico, the largest poultry populations are located in a huge plateau at an altitude in excess of 1,200 m above sea level. Mortality rates attributable to AS in this region can be as high as 20%

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of the total broiler population,² although more conservative figures also have been reported.^{3,4}

The easiest, most popular method used to prevent AS on these farms in Mexico consists of limiting feed intake. Disadvantages of this practice include decreased growth rates, which in turn lead to lower profits. As a whole, AS and limited growth rates that result from feed restriction are the number 1 cause of economic losses in most of Mexico's poultry regions.

Ascites syndrome is characterized by hypoxia attributable to an imbalance between fast muscular growth and the limited cardiopulmonary development of broilers, which results in increased pulmonary arterial pressure, general hypoxemia, pulmonary edema, and death.⁵ Large numbers of inflammatory cells are found in several tissues,⁶ which result in high concentrations of free radicals.⁷ Typically, the liver and heart of broilers with clinical signs of AS are affected.⁸ Free radicals may oxidize carbohydrates, lipids, proteins, and nucleic acids, thus causing a worsening of the disease condition in poultry.⁹

The use of antioxidants such as vitamins C or E may be an approach to prevent or alleviate AS in fast-growing broilers. Particularly, vitamin E is an antioxidant that scavenges free radicals released from damaged membranes.^{10,11} The use of vitamin E for this purpose has been reported elsewhere.^{12,13} In ascites induced by reducing ventilation, vitamin E implants decreased the cumulative mortality attributable to AS, reversed the decrease in glutathione concentrations in the liver, and decreased plasma concentrations of lipid peroxide.¹³ Abundant supplementation of vitamin E (300 to 500 mg/kg of diet) may alleviate peroxidation of the liver and cell damage caused by free-oxygen radicals typically found in heat-stressed poultry.¹⁴ On the other hand, supplementation of diets with up to 87 mg of DL- α -tocopherol acetate did not have an effect on growth performance (body weight, feed intake, or feed efficiency).¹⁰ The purpose of the study reported here was to evaluate whether dietary supplementation with vitamin E or vitamin C would affect oxidative stress on the heart, growth performance, and cumulative mortality attributable to naturally developing AS in broiler flocks maintained under commercial conditions at an altitude of approximately 2,200 m above sea level on farms located in the vicinity of Mexico City.

Materials and Methods

Animals—Young (1 day old) broilers (n = 360) purchased from a commercial source^a were used in the study.

Broilers were weighed and randomly assigned to 3 dietary treatment groups (120 birds/group). Birds were housed for the duration of the 7-week study in 9 pens (40 birds/pen) in environmentally controlled rooms at an altitude of approximately 2,235 m above sea level.

Diets—Birds were provided standard starter and grower diets for broilers that had been formulated to fulfill nutritional requirements.¹⁵ Birds in the control group received a basal diet that contained 12 mg of DL- α -tocopherol acetate (ie, vitamin E)/kg of feed without additional ascorbic acid (ie, vitamin C). Birds in the vitamin E group were fed the basal diet supplemented with 75 mg of DL- α -tocopherol acetate/kg of feed without additional ascorbic acid. Birds in the vitamin C group were fed the basal diet supplemented with 400 mg of ascorbic acid/kg of feed without additional DL- α -tocopherol acetate. Feed and water were provided ad libitum throughout the study.

Experimental design—Cumulative body weight, feed consumption, and feed conversion rates (kilograms of feed intake per kilograms of body weight gained) were determined for each group on a weekly basis. Nine randomly selected birds from each group were euthanatized every week; remaining birds in each group were euthanatized at the completion of the 7-week study. Birds were euthanatized by cervical dislocation. Immediately after birds were euthanatized, samples of cardiac tissues were obtained, minced, and placed on tissue paper to remove excess blood. Samples then were frozen at -70 C until analysis.

Analysis of cardiac tissues—Concentration of thiobarbituric acid reactive substances (TBARS) was assessed by use of the method described by Zentella de Piña et al.¹⁶ Briefly, homogenates of cardiac tissues were filtered through a cheesecloth, and an aliquot of the filtered homogenate was incubated with 1.0 ml of 0.15M phosphate buffer (pH 7.4) for 30 minutes at 37 C. Then, 1.5 ml of 20% acetic acid (pH 2.5) and 1.5 ml of 0.8% thiobarbituric acid were added. The mixture was placed in boiling water for 45 minutes. Tubes were allowed to cool, and 1 ml of 2% KCl and 5 ml of butanol:pyridine (15:1) solution were added to each tube. Tubes were mixed vigorously, and absorbance of the organic layer was measured, by use of a spectrophotometer at a setting of 532 nm. Concentration of TBARS in the samples was calculated, by use of an extinction coefficient of $1.56 \times 10^5/M/cm$.¹⁷ Protein concentration was determined, by use of the method described by Bradford.¹⁸

Statistical analysis—A 1-way ANOVA was used to com-

pare differences in variables among and within groups of various ages, using a statistical analysis program.¹⁹ Values of $P < 0.05$ were considered significant.

Results

Broilers—Clinical signs of AS were not detected during the study. Overall mortality (mean \pm SEM, 20.0 \pm 2.7%) and mortality attributable to AS (16.1 \pm 2.6%) were not affected by feeding vitamin-supplemented diets.

Growth rate—Results for growth rate were divided into 2 stages (stage 1, which included the first 3 weeks of dietary treatment, and stage 2, which included weeks 4 to 7 of dietary treatment). Results of the entire 7-week study also were determined. Data for feed consumption, growth rate, and feed conversion of all 3 groups were summarized (Table 1).

Differences were not observed between vitamin C and vitamin E groups regarding feed consumption, growth rate, and rate of feed conversion, compared with values for the control group during stage 1 (Table 1). On the other hand, during stage 2 and for the entire 7-week period, feed consumption was significantly decreased in the group fed a diet supplemented with vitamin C, compared with feed consumption for the control group. During stage 2, growth rate was improved for birds fed either of the vitamin-supplemented diets. Overall, feeding of vitamin-supplemented diets improved growth rate during the entire 7-week study. The best growth rates were recorded for broilers fed the diet supplemented with vitamin E; growth rates for these broilers were significantly better during the 7-week study, compared with values for the control and vitamin C groups. Rate of feed conversion was significantly lower in the vitamin E group for stage 2 and the entire 7-week study and in the vitamin C group for the entire 7-week study, compared with the control group.

Concentration of TBARS—The TBARS concentration in cardiac tissues of broilers euthanatized weekly throughout the study was measured (Fig 1). Lower TBARS concentrations were detected in broilers fed diets supplemented with vitamins E or C. Dietary sup-

Table 1—Mean (\pm SEM) values for feed consumption, growth performance, and rate of feed conversion in broilers fed a basal (control) diet, the basal diet supplemented with vitamin E, or the basal diet supplemented with vitamin C

Variable	Time during study (wk)	Control	Vitamin E	Vitamin C
Feed consumption (g)	0 to 3	910 \pm 3.8	921 \pm 27.3	885 \pm 9.3
	4 to 7	3,922 \pm 21.9 ^a	3,858 \pm 31.8 ^{ab}	837 \pm 50.2 ^b
	0 to 7	4,832 \pm 21.5 ^a	4,779 \pm 11.1 ^{ab}	4,722 \pm 55.0 ^b
Growth performance (g of body weight)	0 to 3	616 \pm 3.0	622 \pm 4.8	627 \pm 4.2
	4 to 7	1,831 \pm 8.3 ^a	1,948 \pm 21.4 ^{ab}	1,862 \pm 29.2 ^a
	0 to 7	2,447 \pm 8.8 ^a	2,571 \pm 16.9 ^a	2,489 \pm 26.6 ^a
Feed conversion (kg of feed/kg of body weight gained)	0 to 3	1.48 \pm 0.00	1.48 \pm 0.03	1.41 \pm 0.03
	4 to 7	2.14 \pm 0.01 ^c	1.98 \pm 0.01 ^d	2.06 \pm 0.01 ^{c,d}
	0 to 7	1.98 \pm 0.00 ^c	1.86 \pm 0.02 ^d	1.89 \pm 0.00 ^d

^{a-d}Within each row, values with different superscript letters differ significantly (^{ab} $P < 0.05$; ^{c,d} $P = 0.01$).

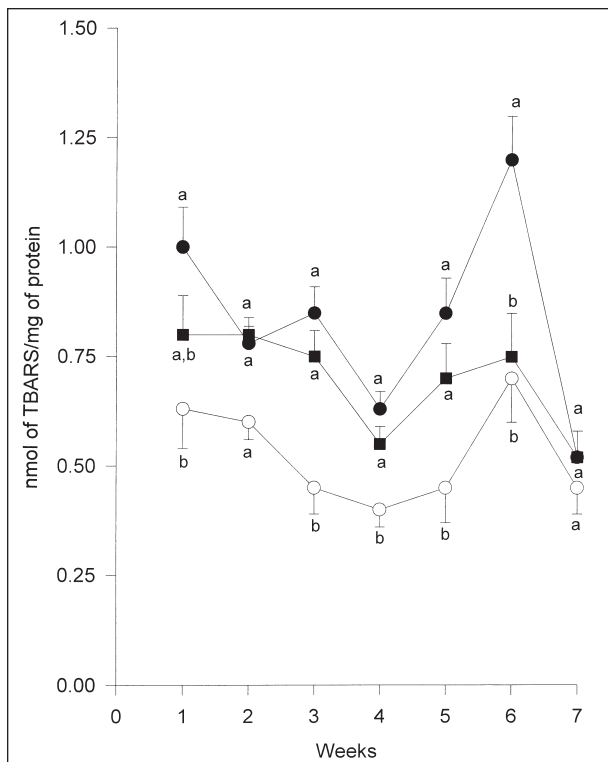


Figure 1—Mean \pm SEM values for lipid peroxidation measured as concentration of thiobarbituric acid reactive substance (TBARS) in cardiac tissues obtained weekly from broilers being fed a basal (control) diet (solid circle), the basal diet supplemented with vitamin C (solid square), or the basal diet supplemented with vitamin E (open circle). There were 9 birds in each group at each time point. ^{a,b}For each week, values with different superscript letters differ significantly ($P < 0.05$).

plementation with vitamin E resulted in significantly lower TBARS concentrations in 5 of 7 weeks (weeks 1 and 3 through 6), compared with concentrations in the control group, whereas dietary supplementation with vitamin C caused significantly lower concentrations only in week 6, compared with concentrations for the control group.

Discussion

Some differences between vitamin E supplementation of broiler diets reported elsewhere and in the study reported here should be clarified. In other studies, the experimental design included specific conditions that favored heat stress¹³ or development of pulmonary hypertension syndrome in birds.¹ In the study reported here, our objective was to prevent or alleviate oxidative stress and the possibility of AS in broilers maintained under strict commercial conditions at 2,200 m above sea level. We decided to feed the flock throughout the entire 7-week period without restricting caloric intake; restriction of caloric intake to prevent AS is a common practice in Mexico.² Amounts of vitamin E used in our study were identical to those used by Bottje et al.¹⁰ (87 mg/kg of diet) but lower than those reported by Williams¹⁴ (300 to 500 mg/kg of diet). There is general agreement that the addition of vitamin E to balanced feeds results in some improvement of stressed birds. Analysis of results of the study

by Williams¹⁴ suggests that vitamin E may play a role in protecting hens from the effect of heat stress, which would enable them to maintain egg production. Analysis of data reported by Bottje et al.¹⁰ indicates that diets supplemented with α -tocopherol acetate exert a dose-dependent response on hepatic and pulmonary α -tocopherol concentrations. However, supplementation of the diets in that study did not have an effect on growth performance in broilers, and supplementation was ineffective in decreasing mortality attributable to pulmonary hypertension syndrome. Analysis of results of the study reported here indicates that diets supplemented with vitamin E resulted in better growth performance, lower rates of feed conversion, and lower TBARS concentrations in cardiac tissues of broilers (Fig 1; Table 1). Our results on growth performance differ from those reported by Bottje et al.¹⁰ Given that the amount of tocopherol acetate added to the diets was the same, differences must be attributed to the experimental conditions used to promote oxidative stress in the birds of each study. Bottje et al.¹⁰ induced stress by decreasing temperature, reducing ventilation, and increasing amounts of air dust, which caused a 2-fold increase in ammonia concentrations. In our study, we assumed that a 10% decrease in atmospheric oxygen availability at an altitude of 2,200 m was the main promoter of oxidative stress. This seems to be a moderate yet constant deleterious environmental condition. Similar to results of another study,¹³ we were unable to affect cumulative mortality.

Results for supplementation with vitamin C included lower feed consumption, lower rates of feed conversion, and a decrease in TBARS concentrations in cardiac tissues of broilers (Fig 1; Table 1). One attractive alternative for future experiments may be the supplementation of diets with both vitamins. It is tempting to recommend that dietary supplementation with vitamin C, vitamin E, or both be used to increase the growth performance of broilers maintained on farms at nearly 2,000 m of altitude, which is the situation for much of the Mexican Republic. Nonetheless, a detailed economic analysis is required to ensure the correct decision is implemented. Thus, it is possible that dietary supplementation with vitamin E will be limited to birds that are 4 to 7 weeks old to maintain low feed costs.

^aArbor Acres \times Arbor Acres strain, Arbor Acres Farms Inc, Mexico City, Mexico.

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