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Ascorbic Acid and Citric Flavonoids for Broilers Under Heat Stress: Effects on Performance and Meat Quality

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■ Keywords

Ascorbic acid, broiler, heat stress, flavonoids.

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ABSTRACT

The aim of this study was to evaluate the effects of increasing doses of ascorbic acid (AA) and citric flavonoids (quercetin and rutin) on the performance and meat quality characteristics of broilers submitted to cyclic heat stress. Four-hundred one-day-old female Ross 308 were housed in 40 battery cages a in temperature controlled room. Treatments consisted of 0, 250, 500, and 1000 g/ton on of AA + citric flavonoids. Birds were fed ad libitum until 32 day of age. Beginning on day 14 post-hatch until the end of the experiment, in order to simulate cyclic heat stress, the temperature inside the room was increased to 32°C for 5 hours, and decreased until reaching the comfort temperature corresponding to the age of the animals. Birds were slaughtered at 33 days of age, and carcass and commercial cuts yields were determined. Thighs and boneless breast samples were collected and frozen for subsequent analyses of pH, cooking loss, shear force, color, and Thiobarbituric Acid Reactive Substances (TBARS). Significant differences ($p < 0.05$) were found for feed efficiency from 1 to 7 days of age, with the best values for the birds fed 0 and 250 g/ton on of AA + citric flavonoids. At the end of the experiment, there were no differences in other performance variables, carcass and parts yields, pH, shear force, color and TBARS. The meat of the birds supplemented with 250 g/ton on of product presented the lowest cooking loss.

INTRODUCTION

Poultry production in tropical countries is affected by the combined effects of high environmental temperature and humidity during most month of the year. As the ratio between humidity and environmental temperature exceeds the thermal comfort zone, birds become more susceptible to heat stress, which negatively influences performance and the efficiency of the immune system (Thaxton & Siegel, 1970; Borges *et al.*, 2003).

During stress, body and immune system cells undergo enzymatic or self-oxidative peroxidation through actions that involve free radicals, oxygen-reactive species, resulting in destabilization of the cell wall, and consequently, cell function loss (Puthpongsiripom *et al.*, 2001). Excessive levels of free radicals may lead to lipid peroxidation, leading to several pathologies in the animals, and affecting meat organoleptic characteristics (Silva *et al.*, 2002; Fellenberg & Speisky, 2006).

The use of several dietary alternatives has been recommended to alleviate of high environmental temperature impact, such as changes in feeding and drinking water management. In addition, dietary nutritional changes, including replacing carbohydrates by lipids or reducing crude protein level, and the use of salts and additives in the drinking water have shown positive effects (Dale & Fuller, 1978; Mendes *et al.*, 1997; Borges *et al.*, 2003).

The use of traditional antioxidants, such as ascorbic acid (AA) has been recommended, and, in some cases, has presented promising effects in the attenuation of heat stress on performance (Stilborn *et al.*, 1988). Alternatively, some compounds with antioxidant activity, such as flavonoids, may also be beneficial. Flavonoids (quercetin and rutin) are obtained by the fermentation of citrus, and polyphenolic substances widely distributed in plants. Many biological effects have been attributed to flavonoids as they may act as antioxidants in the liver and protector of blood vessels (Silva *et al.,* 2002; Tieppo, 2007). According to Bianchi & Antunes (1999), the combined use of antioxidants can reduce the effects of stress due to its oxi-reduction properties, which may have an important role in free radical quenching and neutralization, in addition to improving meat organoleptci characteristics and quality (Peterson & Dwyer, 1998).

The present study aimed at evaluating the effect of increasing AA and citric flavonoid doses on live performance and meat quality traits of broilers submitted to cyclic heat stress.

MATERIAL AND METHODS

A total number of 400 female Ross 308 broilers (44 ± 0.5 g) were used. Birds were distributed in randomized block design, with four treatments of 10 replicates of 10 birds each. The experiment was carried out in a temperature-controlled room. Birds were housed in metal cages (0.80m x 0.90m) equipped with a feeder and a drinker each. Mash feeds were formulated with corn and soybean meal according to the recommendations of Rostagno *et al.* (2005), and are shown in Table 1. Treatments consisted of diets containing graded levels of AA and citric flavonoids, as presented in Table 2. The commercial product Biocitro® (Quinabra, São José dos Campos, Brazil), obtained by citrus fermentation, was used. It contains 10.0% AA and 0.7% flavonoids (50% quercetin and 50% rutin). The feeding program was divided in two phases: starter, fed from 1 to 20 days, and grower, fed from 21 to 32 days.

Performance was weekly assessed, measuring body weight, feed intake, feed conversion ratio, and mortality. Initial room temperature was 32° C, which was then daily adjusted until 25° C at 13 days of age. From day 14 until the end of the experimental period, room temperature simulated cyclic heat stress: it remained 32° C for 5 hours, and then was slowly reduced (1° C every 12 min) until reaching the comfort temperature that corresponded to bird age.

Broilers were slaughtered at 33 d of age after 8 hours of fasting. Carcasses were chilled according the official regulations (Regulamento Técnico de Inspeção Tecnológica e Higiênico Sanitária de Carne de Aves; Brasil, 1998). Carcasses were then processed, and the following cuts were produced: deboned breast meat, breast fillet, thighs, legs, wings, and back. Breast and thighs were individually packaged in gas-impermeable plastic film (BB300-Cryovac®), and stored in a freezer at -18° C for 230 days. All cuts were individually weighed and expressed relative to empty carcass weight.

Table 1 – Composition of the starter (1-20 days) and grower (21-32 days), %.

Ingredients	Initial	Grower
Corn	57.32	59.25
Soybean meal	36.35	32.80
Soybean oil	2.37	4.38
Calcitic limestone	0.90	0.83
Dicalcium phosphate	1.75	1.61
Salt	0.34	0.35
Sodium bicarbonate 99%	0.09	0.00
Biolys 50.7% Lys	0.32	0.26
DL-Methionine 99%	0.27	0.23
L-Threonine	0.07	0.05
Choline chloride, liq.	0.05	0.06
Mineral Premix Broiler	0.05	0.05
Vitamin Premix Broiler	0.12	0.12
TOTAL	**100.00**	**100.00**
Nutritional levels, % or as described		
AMEn, kcal/kg	3.000	3.150
Crude protein, %	21.6	20.0
Lys dig, %	1.20	1.08
Met+Cys dig, %	0.85	0.78
Thr dig, %	0.78	0.71
Val dig, %	0.90	0.84
Ca, %	0.90	0.83
P disp, %	0.44	0.41
Na, %	0.18	0.16
Cl, %	0.30	0.30
K, %	0.90	0.84
BED, mEq/kg	224	200
Cholin, mg/kg	1.600	1.600

Enrichment per kg product: Vit A: 10,000,000 IU; Vit D3: 3,000,000 IU; Vit E: 40,000 IU; Vit K3: 3,000 mg; Vit B1: 2,000 mg; Vit B2: 6,000 mg; Vit B6: 4,000 mg; Vit B12: 20,000 mg; Nicotinic acid: 40,000 mg; Pantothenic acid: 12,000 mg; Folic acid: 1,000 mg; Biotin: 150 mg; Selenium: 250 mg; Fe: 60,000 mg; Cu: 10,000 mg; Zn: 100,000 mg; Mn: 100,000 mg; I: 1,000mg.

Breast meat pH, cooking loss (Chrystall *et al.,* 1994), and shearing force (Liu *et al.*, 2004) were determined. Meat color was measured in the center of the *Pectoralis major* (internal face) using a MINOLTA Chroma Meter CM 508-d spectrometer. L*, a*, and b* parameters were read according to the ICE (International Commission on Illumination) system. Thigh samples were submitted to TBARS assessment (Tarlagdis *et al.*, 1960).

Table 2 – Concentration of the commercial product and active principle, according to the treatments (g/ton).

Treatment	Commercial product[1]	Active principle concentration	AA	Quercetin 50% Rutin 50%
Negative control	0	0	0	0
T1	250	87.5	8.75	0.61
T2	500	175	17.50	1.22
T3	1000	350	35.00	2.45

1 - Biocitro®, commercial product obtained by the fermentation of citric fruits; contains 10.0% AA and 0.7% flavonoids.

Data were submitted to analysis of variance and regression using SAS (2001) package, and means that presented statistical difference were compared by the test of Tukey at 5%.

RESULTS AND DISCUSSION

Live performance: No treatment effect was detected on body weight, feed intake, or mortality (p≥0.05) (Table 3). General mortality was high, above the values commonly found under commercial production conditions. In the absence of health problems, this was probably consequence of the heat stress imposed to birds in all treatments.

Table 3 – Body weight (BW, g), weight gain (WG, g), feed intake (FI, g), and mortality (M, %) of one- to 32-day-old female broilers.

Treatments g/tonon	BW[1]	WG[2]	FI[3]	M[4]
0	1640	1597	2462	17.0
250	1620	1576	2443	17.0
500	1629	1584	2467	13.0
1000	1606	1561	2451	10.0
CV, %	3.6	3.7	3.4	48.3
PROB	>0.601	>0.590	>0.913	>0.826

1 - Y=1.64+(-0.00003)X; p<0.0001; R^2=0.04; 2 - Y=1.53+(-0.00003)X; p<0.0001; R^2=0.043; 3 - Y=2.45+(-0.000004)X; p<0.0001; R^2=0.0004; 4 - Y=17.60+(-0.0076)X; p<0.0001; R^2=0.029.

Feed conversion ratio was statistically different (pd<ˆ 0.001) for the period of 1 to 7 days of age, with broilers fed 0 and 250 g/ton Biocitro® presenting better feed conversion ratio as compared to those fed 500 and 1000 g/ton (Table 4).

The lack of treatment effects maybe related to the difficulty to determine the degree of stress to which the birds are experimentally submitted (Mckee & Harrison, 1995; Whitehead *et al.*, 2003). Puron & Santamaria (1994) did not observe differences in weight gain, feed intake, feed conversion ratio or mortality of broilers fed 200 ppm AA, and attributed these results to the temperatures and times applied (35-38° C for 4 hours and 26-34° C for 6 hours, respectively), as the beneficial effects of AA and flavonoids supplementation are most likely to be observed at higher temperatures. However, cyclic heat stress temperatures and times used in the study of Sahin *et al.* (2003) were higher (34°C for 8 h/d), and resulted in lower feed intake and worse feed conversion ratio in quails fed a diet supplemented with 250 mg L-AA.

Consistent with Vathana *et al.* (2002), favorable temperatures and light provided for the entire 24-h day period may explain the lack of differences in body weight, weight gain, and feed intake among the experimental birds. Birds may have also suffered changes during the acclimation process, and therefore, did not suffer heat stress (Arjona *et al.*, 1988).

AA supplementation showed positive effects on the reduction of corticosterone plasma levels ando n the heterophil/lymphocyte ratio, as evaluated by Mckee & Harrison (1995) in birds submitted to heat stress (33° C) and supplemented with 150 and 300 ppm AA in the diet.

Meat yield and meat quality traits: No statistical differences were detected among the analyzed treatments for carcass and cut yields of 33-day-old birds (p≥0.05) (Table 5).

Table 4 – Feed conversion ratio (g/g) of one- to 32-day-old female broilers.

Treatments g/ton	1-7[1]	7-14[2]	14-20[3]	1-20[4]	20-28[5]	28-32[6]	1-32[7]
0	1.12 [a]	1.33	**1.45**	1.35	**1.72**	**1.71**	1.54
250	1.14 [a]	1.31	**1.45**	1.35	**1.76**	**1.69**	1.55
500	1.15[b]	1.32	**1.47**	1.36	**1.74**	**1.74**	1.56
1000	1.21[b]	1.31	**1.46**	1.36	**1.80**	**1.71**	1.57
CV, %	4.3	3.2	2.3	1.5	**8.5**	**5.3**	2.4
PROB	d 0.001	>0.614	>**0.426**	>0.527	>**0.644**	>**0.673**	>0.364

Means followed by different letters in the same column are different by the test of Tukey at 5% significance. Columns in bold correspond to heat-stress days. 1 - Y=1.12+0.00009X; p<0.0001; R^2=0.37; 2- Y=1.32+(-0.00001)X; p<0.0001; R^2=0.36; 3- Y=1.45+0.00009X; p<0.0001; R^2=0.11; 4 - Y=1.35+0.00001X; p<0.0001; R^2=0.049; 5- Y=1.72+0.00007X; p<0.0001; R^2=0.036; 6- Y=1.71+0.000005X; p<0.0001; R^2=0.0005; 7- Y=1.54+0.00003X; p<0.0001; R^2=0.079.

Table 5 – Carcass, abdominal fat, back, breast, breast fillet, thigh, drumstick, and wing yields of female broilers fed diets supplemented with different concentrations of AA + citric flavonoids, %.

Treatments g/ton	Carcass[1]	Abdominal fat[2]	Back[3]	Breast[4]	Breast fillet[5]	Thigh[6]	Drumstick[7]	Wing[8]
0	77.10	1.62	23.60	24.60	4.87	12.98	19.31	11.97
250	76.60	1.56	23.60	24.40	4.87	13.24	19.01	11.89
500	77.30	1.70	23.60	24.10	4.89	12.92	18.99	11.98
1000	77.20	1.61	23.40	24.20	5.43	12.93	19.06	12.06
CV %	1.9	11.1	2.4	2.4	17.7	2.4	2.9	2.7
PROB	>0.702	>0.345	>0.856	>0.296	>0.422	>0.087	>0.554	>0.727

1 - Y=76.94+0.0*003X*; p<0.0001; R^2=0.007; 2- Y=1.61+0.00002*X*; p<0.0001; R^2=0.0022; 3- Y=23.62+(-0.00014)*X;* p<0.0001; R^2=0.009; 4 - Y=24.44+(-0.0003)*X*; p<0.0001; R^2=0.039; 5 - Y=4.76+0.00057*X*; p<0.0001; R^2=0.06; 6 - Y=13.07+(-0.00014)*X*; p<0.0001; R^2=0.0298; 7 - Y=19.18+(-0.00019)*X*; p<0.0001; R^2=0.017.; 8 - Y=11.92+0.00011*X*; p<0.0001; R^2=0.019.

The use of body energy reserves during heat stress periods may vary with dietary carbohydrate and fat levels or with AA dietary supplementation, as well as with corticosteroid synthesis and the production of thyroid hormones (Dale & Fuller, 1980; Mckee *et al.*, 1997). Although plasma hormone concentrations were not measured in the present study, these probably were not significant as no differences in abdominal fat yield were detected (p>0.05).

Data presented in Table 6 showed that the experimental treatments did not cause any significant differences in breast pH at the different measurement times. However, pH tended to decline as a function of time. After *rigor mortis* resolution (4 h *post-mortem*), breast meat pH is commonly between 5.7 and 5.9, and must remained unchanged after this time. A slight increase is expected after long storage times due to the formation of basic substances (Souza, 2006). As pH was not measured 24 h *post-mortem*, it was not possible to establish if values were lower than those obtained at 4 h. considering that these values were close to those recorded at that time, meat probably presented normal behavior and that birds were not sufficiently stressed to present meat changes, such as those observed in PSE or DFD pork, present in poultry meat with pH higher than 6.2 at 24 h *post mortem or* below 5.7 during the first 45 min after slaughter (Aks'it *et al.*, 2006).

Table 6 – Mean pH values of the breast meat of female broilers fed diets supplemented with different concentrations of AA + citric flavonoids.

Treatments g/ton	1h[1]	4h[2]	Final*[3]
0	6.27	5.97	5.95
250	6.44	5.93	5.90
500	6.30	5.88	5.88
1000	6.37	6.10	5.94
CV %	4.0	3.1	1.3
PROB	>0.454	>0.108	>0.200

* Measurement carried out at thawing (7 months of storage). [1] Y=6.32+0.00004*X*; p<0.0001; R^2=0.004; [2] Y=5.91+0.00012*X*; p<0.0001; R^2=0.053; [3] Y=5.91+(-0.000007)*X*; p<0.0001; R^2=0.0011.

Nevertheless, as shown in Table 7, there were significant differences in cooking loss of thawed weight loss both in grams and in percentage (pd≤0.037 and pd≤0.020, respectively), with the treatment 1000 g/ton promoting the highest loss.

Table 7 – Cooking loss of breast meat samples of female broilers fed diets supplemented with different concentrations of AA + citric flavonoids.

Treatments g/ton	TWL[A1], %	ThWL[B2], g	CW[C3], g	CWL[D4], %	CkWL[E5], g
0	41[ab]	13[b]	221	61	22
250	38[b]	12[b]	217	53	19
500	41[ab]	13[b]	217	57	21
1000	52[a]	17[a]	199	58	22
CV %	24.1	22.2	8.6	19.4	16.3
PROB	d*0.037	d*0.020	>0.066	>0.399	>0.297

Means followed by different letters in the same column are different by the test of Tukey at 5% significance. [A]TWL=thawed weight loss; [B]ThWL= thawed weight loss; [C]CW=cooked weight; [D]CWL=cooked weight loss; [E]CkWL= cooked weight loss.1 - Y=37.10+0.012*X*; p<0.0001; R^2=0.143; 2 - Y=11.66+0.0045*X*; p<0.0001; R^2=0.183; 3 - Y=222.95+(-0.021)*X*; p<0.0001; R^2=0.1655; 4 - Y=57.62+(-0.0009)*X*; p<0.0001; R^2=0.0009; 5 - Y=20.48+0.0014*X*; p<0.0001; R^2=0.019.

These results have no relation with pH values, as the pH highest value seems to indicate a slower decline, leading to higher water retention capacity. According to Souza (2006), breast meat cooking loss varies between 18 and 29%, which is consistent with the results obtained in the present study.

No significant differences were observed in shear force (p>0.05) (Table 8).

However, this parameter has no relation with the results obtained for cooking loss, as meat texture is closely linked to intramuscular water content (Lawrie, 2005). According to Souza (2006), breast meat shearing force values are approximately 2.50 to 5.00 kgf. The low values obtained in the present study could be related to the chemical and centesimal composition of the muscle, which changes with bird age and genetics (Olivo & Olivo, 2005).

Data presented in Table 8 show that the experimental treatments did not result in significant

differences for L*, a*, or b* values. Several authors relate meat color to pH and to water retention capacity (Yang & Chen, 1993; Fletcher, 1995; Moreira, 2005; Olivo & Olivo, 2005). According to Zapata *et al.* (2006), freezing seems to produce a marked darkening, reducing chicken mean luminosity. However, in the present study, results indicate that meat was normal with L* values between 44 and 53, and a pH of 5.7. The a* and b* color components indicate that the meat samples were dark red and yellowish, This was possibly due to partial cell breakdown and blood migration caused by the slow freezing process or to the oxidation of the meat pigment, which stability depends on animal species, muscle biochemical characteristics, and some external parameters (Lyon & Lyon, 2002).

Table 8 – Mean Warner-Bratzler shear force (kgf), L*, a*, and b*, and TBARS (mg malonaldehyde/kg sample) values of breast meat samples of female broilers fed diets supplemented with different concentrations of AA + citric flavonoids.

Treatments g/ton	FC[1]	L*[2]	a*[3]	b*[4]	TBARS[5]
0	1.25	45	3.40	8.30	1.47
250	1.28	45	3.35	8.69	1.58
500	1.61	46	3.31	8.52	1.06
1000	1.40	47	3.21	8.63	1.68
CV %	24.1	4.7	28.1	14.4	40.1
PROB	>0.090	>0.223	>0.971	>0.905	>0.125

1- Y=1.30+0.0002X; p<0.0001; R^2=0.04; 2- Y=44.84+0.002X; p<0.0001; R^2=0.119; 3 - Y=3.40+(-0.00018)X; p<0.0001; R^2=0.0068; 4- Y=8.43+0.0002X; p<0.0001; R^2=0.0051; 5- Y=1.39+0.00011X; p<0.0001; R^2=0.0054.

Table 8 shows mean TBARS levels of the frozen thigh meat samples. Samples of birds fed 250 and 1000 g/ton of the product presented the highest values, and those of treatments 0 and 500 g/ton had the lowest values. However, data analysis did not show any influence of treatments on this parameter.

CONCLUSIONS

The addition of graded AA and citric flavonoids (quercitin and rutin) levels to the broiler diets did not affect live performance, commercial cut yield, carcass yield or meat quality trait parameters. However, differences were found in feed conversion ratio and thawed weight loss. The addition of graded levels of the product impaired feed conversion ratio of birds during the first week of age, and increased breast water loss of birds processed at 32 days of age.

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