The influence of feed supplementation with acid ascorbic and sodium ascorbate on broilers, under thermal stress

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Under stress-conditions the decomposition of ascorbic acid increases, as was shown by the application of ^{14}C-ascorbic acid. There is an increased consumption especially in the leukocytes and the liver. The byosinthesis in stress conditions is not augmented to the necessary degree, so the concentration of ascorbic acid in the plasma is lowered. The administration of ascorbic acid increases the concentration in the plasma and the uptake into the adrenal cortex, into the cells of the immune system and into other cell types.The literature points out that addition of ascorbic acid to the feed of poultry has positive effects, such as increased weight gain, higher resistance to some infections and contagious diseases, more suitable eggshell thickness, better summer egg quality etc. The experiments were conducted with meat chickens, 10 days old, whose feed rations were supplemented with 2 g of ascorbic acid / kg of combined feed, along with another lot receiving 2 g of sodium ascorbate/kg of combined feed, while a third lot received unsupplemented feed. The surrounding temperature was 28 – 32^{0}C, compared to 26 – 22^{0}C, the normal temperature for this age group. After four weeks, blood samples were taken by puncture of the cubital vein and hematologic and biochemical investigations were conducted, as well as dosing of ascorbic acid in tissues and organs. The data were processed biostatistically and plotted by a PC 386 Sx calculation system, using the Lotus R-23 program packet. The significance of difference (t) was then determined. After four weeks supplemented feeding with 2 g of ascorbic acid / kg of combined feed, the average weight of meat chickens was 1035.41 g against only 866.50 g in the controls, hence 19.49% greater. The hematologic and biochemical findings included a drop in the hematocrit ($P<0.05$) and in the amount of ascorbic acid contained in the bursa of Fabricius ($P < 0.01$). In the lot supplemented with 2 g of sodium ascorbate / kg of combined feed, after 4 weeks, the average weight was 977.00 g against only 866.50 g in the control, hence an increase of 12,75%. The hematologic an biochemical findings included a rise in the SO_4 Zn turbidity test values ($P<0.05$), ascorbinemia ($P<0.01$) and the content of ascorbic acid in the kidneys ($P<0.05$), liver ($P<0.05$), and the bursa of Fabricius ($P<0.05$).

Keywords: ascorbic acid; sodium ascorbate; meat chickens; thermal stress; erythrocytic and biochemical blood parameters

Introduction

The biochemical synthesis of ascorbic acid has been studied in animals and in plants. Chickens (Gallus domesticus) can synthesize L-ascorbic acid (AA, vitamin C) in their kidneys (Chaudhuri and Chatterjee, 1969) and they do so in quantities apparently sufficient enough to ensure optimal functioning of their metabolic processes (Scott, 1975). In heart stress studies involving chickens (Pardue et al., 1985), high plasma adrenal steroid levels have been generally associated with plasma electrolyte effects that are indicate of dehydration, increased plasma Na^+ levels and decreased plasma K^+ levels, and elevated plasma protein levels (an indication of tissue protein catabolism). Environmental temperature is one of the major stressors because this can range from -5^0C up to 35 – 43^0C, for prolonged periods. The ideal temperature for broilers is 10 – 22^0C for optimum body weight and 15 – 27^0C for feed efficiency (Rama Rao et al., 2002). In the temperature above 30^0C, performance will be depressed in terms of growth, feed intake etc., and mycotoxins develop very quickly in hot and humid conditions, leading to loss of calitity production, immunosuppression and higher mortality (Curcă, 1993; Curcă et al., 1998, 1999, 2003).

The literature points out that addition of ascorbic acid (AA) to the feed of poultry has positive effects, such as increased weight gain, higher resistance to some infectious and contagious diseases (Gross, 1988), more suitable eggshell thickness (Kechick et al., 1974), better egg quality etc.

Materials and methods

The experiments were conducted with meat chickens, 10 days old, whose feed rations were supplemented with 2 g of ascorbic acid/kg of feed, along with another lot receiving 2 g of sodium ascorbate/kg of feed, while a third lot received unsupplemented feed. The surrounding temperature was 28-32^0C, compared to 26-22^0C, the normal temperature for this age group. After four weeks, blood samples were taken by puncture of the cubital vein and hematologic and biochemical investigations were conducted (Kondi,1981; Manta et al.,1976) as well as dosings of ascorbic acid in tissues and organs (Roe, 1943).

Results and discussion

After 4 weeks of supplementing feeding with 2 g of ascorbic acid/kg of combined feed, the recorded weight gain amounted to 19, 49%, that is the average weight was 1035.41 g against only 866.50 g in the controls. Direct as well as derived erythrocyte parameters did not show significant change statistically; nevertheless we need to observe a decreasing tendency of the hematocrit by 15.25% as compared to the one in control, probably due to water retention in the vascular system; as a result of hemoglobin genesis stimulation a rise by 8.55% in the M.C.H.C. level has been observed, if the average with the experimental batch was 20.388 ± 0.766 g Hb/dl RBC, the control values amounted to 18.779 ± 0.221 g Hb/dl RBC (Table 1). Four weeks after the feed rations were supplemented by 2 g sodium ascorbate/kg of combined feed, the average weight was 977.00 g, that is 12.75% greater than the that of the control batch, whereas the direct and derived erythrocyte parameters analysis did not show a significant change statistically; however, we need to observe a drop by 13.93% in erythremia (RBC), by 9.37% in hemoglobinemia, by 12.71% in the hematocrit, by 7.78% in the M.C.V. while the M.C.H.C. values have increased by 6.46% as compared to the control batch. When the thyroid secretion is clearly dependant on the surrounding temperature levels (Kechik et al., 1974; Kolb, 1984; Lahann, 1970), this will significantly impact a number of functions among which weight gain and egg production (Kechik et al., 1974; Perek, 1984), as well as the intermediate metabolism, more specifically the amino acids metabolism and that of some macro- and microelements: iron, copper, selenium, lead

and mercury (Tagwerker, 1960). The synergy implied by the presence of ascorbic acid within the iron metabolism as well as in the hemoglobin genesis is universally acknowledged while the chemical reactions between copper and ascorbic acid are antagonistic.

Table 1. The values of some direct and derived erythocytic parameters in meat chickens under thermal stress, after administration of ascorbic acid and sodium ascorbate

Lot	Statistical parameters	Direct erythrocytic parameters			Derived erythrocytic parameters		
		Erythremia x 10^6/µl blood	Hemoglobinemia g/dl blood	Hematocrit (PCV) %	M.C.V. μ^3 RBC	M.C.H. pg Hb/RBC	M.C.H.C. g Hb/dl RBCs
I Control lot	$\bar{X} \pm s_{\bar{x}}$	3.533 ± 0.232	5.911 ± 0.239	31.483 ± 1.424	90.576 ± 6.438	17.036 ± 1.689	18.779 ± 0.221
	Range	3.200 – 4.100	5.509 – 6.565	29.410 – 34.950	73.390 – 109.21	13.436 – 20.516	18.306 – 19.245
	s	0.403	0.438	2.487	14.663	2.892	0.383
II Ascorbic acid	$\bar{X} \pm s_{\bar{x}}$	3.167 ± 0.055	5.408 ± 0.159	26.667 ± 1.476	84.061 ± 3.265	17.08 ± 0.464	20.388 ± 0.766
	Range	3.100 – 3.300	5.018 – 5.612	23.950 – 30.090	77.258 – 91.182	16.187 – 18.135	18.557 – 21.650
	s	0.094	0.278	2.556	5.689	0.803	1.326
t (significance difference)		n.s.	n.s.	x	n.s.	n.s.	n.s.
III Sodium ascorbate	$\bar{X} \pm s_{\bar{x}}$	3.100 ± 0.125	5.421 ± 0.407	27.493 ± 2.902	89.327 ± 10.165	17.528 ± 1.253	19.994 ± 1.159
	Range	2.800 – 3.300	4.867 – 6.415	21.360 – 33.670	64.727 – 105.218	14.748-20.046	18.146 – 22.78
	s	0.218	0.704	5.028	17.640	2.171	2.008
t (compared with control lot)		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
t (compared with ascorbic acid)		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Differences: n.s. = nonsignificant difference (P>0,05); x = significant difference (P<0,05).

The level of some of the biochemical blood parameters in broilers following ascorbic acid administration and subjected to thermal stress has not shown significant change statistically; however, we need to observe an increasing tendency in protein levels by 12.60%, in the SO_4Zn turbidity test values by 47.60% and 35.00% respectively, and a drop in pyruvicemia by 7.35% as compared to the control batch (Table 2). A statistically significant drop in turbidity test values ($P<0.05$) were recorded in the chickens whose feed rations was supplemented with sodium ascorbate together with an important rise in ascorbinemia ($P<0.01$). The rise in turbidity test values is due to a drop in albumin levels and a rise in globulin levels respectively, situation achieved by means of a stimulating activity on antibody genesis processes by intensifying the lympho-genesis function, especially that of the spleen and of the Fabricius bursa; on the other hand, due to an increased growth rate/speed, there is a intensified consumption of plasmatic albumin which is a readily used source. The supplementary ascorbic acid given in the combined feed cuts down on the incidence of *E.Coli* from 76% in chickens whose feed was not supplemented, to 19% in chickens whose feed rations was supplemented by 330 mg/kg (Gross, 1988), and maintains the heterophile/lymphocyte balance which drops under stress to 0.33. Some very important aspects were recorded when analyzing the ascorbinemia and the content of ascorbic acid levels in tissues and organs that have a bearing on understanding the metabolic peculiarities of the ascorbic acid in chickens. The chicken batch whose feed was supplemented by ascorbic acid recorded a rise in ascorbinemia levels by 32.42% as compared to the control batch, yet statistically insignificant ($P>0.05$), whereas the batch whose feed was supplemented by sodium ascorbate recorded a significant rise ($P<0.01$) (Table 3). There has been a statistically insignificant rise in the ascorbic acid content in the adrenals with the batch whose feed was supplemented by 2 g of ascorbic acid/kg of combined feed, while the batch whose feed was supplemented by sodium ascorbate recorded values under that of the controls' average.

Table 2. The values of some biochemical blood parameters in broilers under thermal stress, after administration of ascorbic acid and sodium ascorbate

Lot	Statistical parameters	Glycemia mg/dl	Proteinemia g/dl	Turbidity test SO_4Zn		Pyruvicemia mg/dl	Ascorbinemia mg/dl
				U.T.	U.T./mg protein		
I Control lot	$\bar{X} \pm s_{\bar{X}}$ Range s	203.84 ± 1.98 200.00 – 208.33 3.43	3.21 ± 0.18 2.86 – 3.62 0.32	5.53 ± 0.38 4.80 – 6.40 0.66	1.73 ± 0.06 1.54 – 1.88 0.14	4.58 ± 0.18 4.24 – 5.00 0.31	3.06 ± 0.19 2.57 – 3.33 0.34
II Ascorbic acid	$\bar{X} \pm s_{\bar{X}}$ Range s	202.35 ± 7.43 192.30 – 220.51 12.87	3.26 ± 0.26 2.62 – 3.63 0.45	6.20 ± 0.71 4.60 – 7.60 1.23	1.89 ± 0.11 1.75 – 2.15 0.19	4.24 ± 0.21 3.85 – 4.73 0.38	4.05 ± 0.56 2.69 – 4.86 0.99
t (significance difference)		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
III Sodium ascorbate	$\bar{X} \pm s_{\bar{X}}$ Range s	195.08 ± 6.00 185.89 – 209.61 10.39	2.98 ± 0.12 2.70 – 3.18 0.21	4.20 ± 0.34 3.40 – 4.80 0.59	1.40 ± 0.07 1.26 – 1.57 0.13	4.55 ± 0.09 4.39 – 4.78 0.16	4.17 ± 0.18 3.73 – 4.42 0.31
t (compared with control lot)		n.s.	n.s.	x	x	n.s.	xx
t (compared with ascorbic acid)		n.s.	n.s.	x	x	n.s.	n.s.

Differences: n.s. = nonsignificant difference (P>0,05); x = significant difference (P<0,05); xx = distinctly significant difference (P<0,01).

Table 3. The ascorbic acid content in organs (mg/100 g fresh tissue) in broilers under thermal stress, after administration of ascorbic acid and sodium ascorbate

Lot	Statistical parameters	Ascorbinemia mg/dl blood	Adrenals mg/100 g	Kidneys mg/100 g	Spleen mg/100 g	Liver mg/100 g	Bursa Fabricius mg/100 g
I Control lot	$\bar{X} \pm s_{\bar{X}}$ Range s	3.06 ± 0.19 2.58 – 3.34 0.34	135.84 ± 12.72 115.14 – 116.35 22.03	16.09 ± 1.02 14.08 – 18.40 1.78	42.47 ± 1.65 38.88 – 46.67 3.21	46.22 ± 3.64 37.87 – 53.07 6.29	80.67 ± 3.14 73.00 – 85.00 5.44
II Ascorbic acid	$\bar{X} \pm s_{\bar{X}}$ Range s	4.05 ± 0.56 2.69 – 4.86 0.99	160.99 ± 4.47 154.67 – 171.90 7.74	21.92 ± 2.58 16.53 – 27.47 4.47	46.61 ± 1.24 44.64 – 49.60 2.15	55.95 ± 4.69 45.71 – 65.60 8.13	55.33 ± 2.37 50.00 – 60.00 4.11
t (significance difference)		n.s.	n.s.	n.s.	n.s.	n.s.	xx
III Sodium ascorbate	$\bar{X} \pm s_{\bar{X}}$ Range s	4.17 ± 0.18 3.74 – 4.42 0.31	134.92 ± 2.42 129.63 – 139.89 4.19	22.22 ± 1.08 19.63 – 24.00 1.88	46.19 ± 3.19 41.07 – 53.87 5.53	58.13 ± 1.26 55.47 – 60.80 2.18	69.00 ± 1.70 65.00 – 72.00 2.94
t (compared with control lot)		xx	n.s.	x	n.s.	x	x
t (compared with ascorbic acid)		n.s.	xx	n.s.	n.s.	n.s.	xx

Differences: n.s. = nonsignificant difference (P>0,05); x = significant difference (P<0,05); xx = distinctly significant difference (P<0,01).

Both supplemented batches display a rising tendency in the ascorbic acid content at kidney level with a difference statistically different (P<0.05) to the batch with additional sodium ascorbate in the feed. At spleen level the rise in ascorbic acid content were statistically insignificant; the same was recorded for

liver but the rise was significant in the batch which received additional sodium ascorbate in its feed. Distinctively significant drops were recorded with the Fabricius bursa ($P < 0.01$) in the batch whose feed was supplemented with ascorbic while significant drops ($P < 0.05$) were observed in the batch whose feed was supplemented with sodium ascorbate as compared to the control, due to the cell depletion, the cells are thus directed in the systemic circulation under thermal stress in chickens. Heat effect on the ascorbic acid metabolism in chickens has accounted for a major concern with many a researchers: thus Squibb a. o. (quoted by Tagwerker, 1960) have observed that the ascorbic acid blood levels in 5 week old chickens kept for 10 days during summer in a 37°C temperature increased by 10 or 7% depending on the feeding system as compared to the control batch, whose surrounding temperature was 21°C. Even if the thermal stress operated for a relatively short period of time, it seems that the ascorbic acid levels in the chickens' blood rise with the surrounding temperature, while during winter decrease, reactions contrary to those recorded in mammals which do not synthesize ascorbic acid at liver level.

Clinical observations in hens have shown that during major heat periods a series of symptoms can be removed or seriously diminished by administration of ascorbic acid such as: a drop in weight gain and feed conversion (Baldissera-Nordio, 1957; Pardue et al., 1984; Pardue, 1988; Thornton et al., 1961), decreased resistance of eggshells as well as a decrease in the egg content quality (Kechik et al., 1974; Thaxton et al., 1984).

Thornton (quoted by Tagwerker, 1960) was the first to emphasize that fact that Leghorn chickens react during summer to the supplementary ascorbic acid by an accelerated growth rate; however, contrary to our observations, the broiler chickens do not seem to be affected. Reid (quoted by Tagwerker, 1960) has observed that following an experiment conducted during summer time, which consisted in daily injections of ascorbic acid, the weight gain rose during the first 32 days by 31% in rooster chickens and by 20% in female chickens. In order to explain the mechanism of ascorbic acid under high temperature conditions we started on the assumption that the thyroid activity slows down with the rise of surrounding temperature that subsequently slow down the metabolism. Within this context, feed consumption as well as a series of metabolic functions and circuits is affected (Horning et al., 1978; Nockels, 1984; Pauling, 1970; Perek, 1984). Moreover, the undercurrent diseases together with the impact of conditioned pathogen agents increase the ascorbic acid catabolism so that its plasmatic levels and the various tissue and organ content are seriously diminished (Curcă, 1993; Gross, 1988; Kechik et al., 1974; Kolb, 1984; Sykes, 1978; Tagwerker, 1960). Physiological stress is an enigmatic concept and widely divergent interpretations are commonplace. Recent efforts in our laboratory have concentrated in stress regulatory procedures in chickens, dietary supplementation with AA or sodium ascorbate has produced promising results. After four weeks` supplemented feeding with 2 g of AA/kg combined feed, the hematological and biochemical findings included a drop in the hematocrit ($P < 0.05$) and in the amount of ascorbic acid contained in the bursa of Fabricius ($P < 0.01$). In the lot supplemented with 2 g of sodium ascorbate/kg of combined feed, after four weeks, the hematological and biochemical findings included a rise in the SO_4Zn turbidity test values ($P < 0.05$), ascorbinemia ($P < 0.01$) and the content of ascorbic acid in the kidneys ($P < 0.05$), liver ($P < 0.05$) and the bursa of Fabricius ($P < 0.05$).

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