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Balance of pH of Chicks from Eggs Injected With Ascorbic Acid and Subjected to Thermal Stress

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Abstract. This study aimed to verify that chicks from eggs injected with ascorbic acid and subjected to heat stress would have changes in acid-base balance, compared to chicks incubated at thermoneutral without injection of ascorbic acid. The parameters evaluated were blood pressure of carbon dioxide and oxygen, base excess, total carbon dioxide, concentration of sodium, potassium, ionized calcium, bicarbonate and pH of newly hatched male chicks, hatched from eggs injected with acid ascorbic acid (AA) and subjected to heat stress during incubation. The experimental design was completely randomized in factorial scheme 5 (application levels of ascorbic acid) x 2 (incubation temperatures). The data were subjected to analysis of variance using the General Linear Model procedure (GLM) of SAS ®. For the blood pH was observed significant interaction ($p < 0.05$) between treatments with application in eggs and incubation temperatures. For the other parameters were not significant effects ($p > 0.05$) of AA level and neither temperature of incubation. Analyzing the unfolding of the interaction to pH was observed that chicks from eggs injected with 6% ascorbic acid and subjected to heat stress during incubation had a higher pH value compared with the thermoneutral temperature incubated ($p < 0.05$). Therefore, it is suggested that the incubation of eggs in high temperature (39ºC) can alter the metabolic rate of these embryos.

Keywords. blood, incubation, *in ovo*, thermoneutral

Introduction

Birds under an environmental stress often have their immune function reduced (Miller & Qureshi, 1991). Because of this, many studies have been developed in order to establish the losses in performance of the birds and learn the physiological changes resulting from heat stress and to develop alternatives that minimize the impact of heat on the commercial broiler chicken industry.

Considering that there are procedures that allow the massive inoculation *in ovo* of vaccines without damage to the embryo development. It is reasonable to assume that the exogenous supplementation of broilers embryos can be accomplished using the same technique. If vitamin C really acts as an anti-stress, an imunoactivator and a co-factor for the formation and maintenance of collagen (Spinosa et al., 2002), and hence of the bone, it is possible that in injection of vitamin C would result in the development of stronger broiler chicks.

Materials and methods

An experiment was conducted during the incubation phase, using 500 fertile broiler eggs (Cobb ®), from the same lot of broiler breeder hens, at 47 weeks of age. The eggs were all within the range of 67g ± 2g of weight. The eggs were weighed and distributed homogeneously in four incubators (model IP - 200, Premium Eco), with automatic egg turners (every two hours), control of temperature and monitoring of humidity.

The experimental design was completely randomized in factorial scheme 5 (application levels of AA) x 2 (incubation temperatures). The treatments with application of AA were applied before the incubation: eggs without holes and therefore not injected; and eggs injected in the albumen (100µl) at the following concentrations: 0%, 2%, 4% and 6% of AA. The eggs were incubated in four incubators, two of these at thermoneutral temperature (37.5°C) and two at hot temperature (39°C) for 18 days, after which the incubators had its temperature reduced by 1 ° C until hatching.

After cleaning of the injection site with 100% ethanol, the eggshell was perforated with a sterile needle. Ascorbic acid (Synth, 99% purity) was injected into the albumen, approximately six mm beneath the eggshell. After injection the hole was sealed with a label identifying the lot. The ascorbic acid was diluted using Milli-Q water. This was performed in the dark because of the photo sensitivity of the AA. The glassware used was autoclaved for one hour in order to avoid any contamination.

The evaluated parameters were all in the blood: pressure of carbon dioxide and pressure of oxygen, base excess, total carbon dioxide, concentration of sodium and potassium, ionized calcium, bicarbonate, pH. Analyses were performed in portable clinical analyzer (i-STAT ® Co. - Abbott Laboratories - USA), immediately after collection using a cartridge CG8 ® + (i-STAT ® Co. - Abbott Laboratories - USA), in which drops of venous blood were placed with anticoagulant heparin, to the mark indicated by the cartridge

The obtained data were submitted to analysis of variance by the General Linear Model (GLM) of SAS ® statistical package (SAS Institute, 2002). When significant effects were determined, the comparison of means was performed at 5% probability by orthogonal and polynomial contrasts, as follow: contrast 1 – comparison among treatment 1 versus treatments 2, 3, 4 and 5; contrasts 2, 3 and 4 - using three regression models: linear, quadratic and cubic (Robbins et al., 1979), in order to verify polynomial effects at the levels of ascorbic acid application.

Results and discussion

The results are presented in Table 1. There was no significant effect ($p > 0.05$) of AA acid and temperature on the pressure of carbon dioxide saturation and oxygen, base excess, total carbon dioxide, concentration of sodium and potassium, ionized calcium and bicarbonate. However, for pH, there was significant interaction ($p < 0.05$) among treatments *in ovo* and the incubation temperatures.

TABLE 1. Effect of ascorbic acid injection on pH, carbon dioxide pressure (pCO_2), oxygen pressure (pO_2), base excess, total carbon dioxide (CO_2 total), concentration of sodium concentration of potassium, ionized calcium and bicarbonate from the blood of newly hatched male chicks.

	Evaluated Parameters								
Treatments	pH	pCO_2 (mmHg)	pO_2 (mmHg)	Base excess (mmol/l)	CO_2 total (mmol/l)	Sodium (mmol/l)	Potassim (mmol/l)	Ionized calcium (mmol/l)	Bicarbonate (mmol/l)
					Ascorbic Acid (AA)				
Control	7.43	20.97	19.00	-10.47	15.93	130.00	4.53	0.595	15.07
0%	7.46	19.11	19.60	-10.71	15.14	129.75	3.63	0.320	14.53
2%	7.43	20.27	17.87	-10.71	15.50	129.31	4.32	0.486	14.70
4%	7.47	18.74	18.08	-10.15	15.54	130.92	4.04	0.540	14.77
6%	7.45	19.19	18.78	-11.20	15.13	128.17	4.91	0.360	14.39
					Temperatures (TP)				
Thermoneutral	7.44	20.74	19.03	-10.45	15.93	129.41	4.17	0.483	15.19
Hot	7.46	18.92	18.33	-10.83	15.11	129.56	4.55	0.434	14.31
					Probability for the analysis of variance				
AA	0.6424 ^{NS}	0.2136 ^{NS}	0.8337 ^{NS}	0.9645 ^{NS}	0.8548 ^{NS}	0.1362 ^{NS}	0.2648 ^{NS}	0.3577 ^{NS}	0.9060 ^{NS}
Temperature	0.4346 ^{NS}	0.0891 ^{NS}	0.7184 ^{NS}	0.8522 ^{NS}	0.5107 ^{NS}	0.7539 ^{NS}	0.2816 ^{NS}	0.4384 ^{NS}	0.4470 ^{NS}
Interaction AA x TP	0.0071*	0.0939 ^{NS}	0.0925 ^{NS}	0.0618 ^{NS}	0.0748 ^{NS}	0.7025 ^{NS}	0.8730 ^{NS}	0.1602 ^{NS}	0.0736 ^{NS}
Coeficient of variance (%)	0.96	16.74	20.62	-36.32	20.61	2.28	32.59	40.55	21.33

^{NS} not significant, * significant at the level of 5% of probability.

Table 2 shows data for the unfolding of the interaction between the levels of AA and incubation temperatures for the blood pH of chicks. The chicks injected with 6% ascorbic acid and subjected to hot incubation during had a higher pH value ($p < 0.05$) compared with the pH of chicks hatched from eggs incubated at thermoneutral.

TABLE 2. Unfolding of the interaction for the blood pH.

	pH		
Treatments	Temperatures (TP)		Probability of the means test
Ácido ascórbico (AA)	Termoneutra	Quente	
Controle	7.45	7.42	0.4167 ^{NS}
0%	7.44 **	7.47 **	0.5279 ^{NS}
2%	7.47 **	7.40 **	0.0694 ^{NS}
4%	7.46 **	7.48 **	0.7212 ^{NS}
6%	7.39 b **	7.52 a **	0.0012*
	Probabilidade para os contrastes		
Control vs Levels	0.7378 ^{NS}	0.1044 ^{NS}	
Linear effect for the levels	0.3720 ^{NS}	0.1168 ^{NS}	
Quadratic effect for the levels	0.1515 ^{NS}	0.0429*	
Cubic effect for levels	0.8186 ^{NS}	0.1015 ^{NS}	

^{NS} not significant, * significant at 5% probability, a-b comparison in the line . ** mean statistically equal to the control by Tukey test at 5% probability.

Researches has been conducted to better understand the influence of temperature on embryo development. One of the factors involved seems to be the availability of oxygen to the embryo. If the embryo temperature increases, so does their metabolism, requiring more oxygen at the end of incubation. If this demand is not met, more negative consequences of high temperature are observed than when there is sufficient oxygen available (Lourens et al., 2007).

Therefore, it is suggested that if the incubation of the eggs in higher temperature alters the metabolic rate of these embryos, the respiratory system of these birds needs to adapt to counteract possible changes in pulmonary ventilation, thus modifying blood pH in order to restore normal conditions.

There was a quadratic effect of the application levels of AA *in ovo* ($p < 0.05$), for the blood pH broiler chicks hatched from eggs incubated at a warm temperature (Table 1). According to the equation $pH = 0{,}0069x^2 – 0{,}0297x + 7{,}4605$ ($R^2=0{,}7585$) it was observed that the lowest values were obtained for broiler chicks from inoculated eggs with 2% AA.

In the comparison among treatments, for both temperatures, there were no significant differences (p> 0.05) levels of AA application, when compared with the control at different incubation temperatures.

Conclusion

The results showed that application of ascorbic acid *in ovo* did not affect embryo development, but there were influences of incubation temperature on embryonic metabolism.

Acknowledgements

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