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Blood Parameters of Chicks from Eggs Injected With Ascorbic Acid and Subjected to Thermal Stress

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***Abstract*.** This study was aimed to verify if chicks from eggs injected with ascorbic acid and subjected to thermal stress would have higher immunity than chicks from incubation at thermoneutrality without injection of ascorbic acid. The parameters evaluated were temperature on oxygen saturation in hemoglobin, glucose, number of erythrocytes, hematocrit rate and number of hemoglobins of newly hatched male chicks, hatched from eggs injected with ascorbic acid (AA) and subjected to thermal 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 parameters (number of erythrocytes, rate of hematrocit and values of hemoglobin), there was significant interaction ($p < 0.05$) between treatments in egg and incubation temperatures. Analyzing the interactions for these parameters, it was observed that the application of 0% ascorbic acid in egg minimized the effect of heat stress when compared with treatment without injection. The application of ascorbic acid levels in eggs incubated under heat stress failed to maximize the immunity of newly hatched chicks. It is assumed that the increased liquid in the amniotic fluid, in those embryos injected with water, favored the lower heat conductance for these embryos, thus helping in their development in relation to immunity. Considering that hemoglobin is related to the transport of gases, these data suggest that increasing the concentration of AA solution inoculated may influence the respiratory rates of eggs.

Keywords. birds, broiler chicken, immunity, incubation, *in ovo*

Introduction

Temperature may be considered the physical factor of greater effect on performance of broiler chickens, with great influence on feed intake (Teeter et al., 1984), weight gain and feed conversion. Animals under stress exhibit metabolic changes that can be noticed by biochemical and hematological changes (Miller and Qureshi, 1991). Given this situation, many studies are developed in order to establish the damage on broiler performance, know the physiological changes resulting from heat stress and to develop alternatives that minimize the impact of heat on commercial broiler flocks.

Thornton (1961) reported that the levels of ascorbic acid in blood of heat stressed birds was markedly reduced, suggesting that the animals under heat stress could receive supplemental vitamin C. Performance characteristics and immune function of birds suffering heat stress are significantly improved with increasing levels of vitamin C (Pardue et al., 1985).

Considering the above, this study aims to verify that broiler chicks from eggs injected with ascorbic acid (AA) and subjected to heat stress, present increased immunity, development and higher post-hatching performance than the broiler chicks hatching from thermoneutral temperature without injection of AA.

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, 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 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 AA 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 parameters evaluated were temperature of oxygen saturation of hemoglobin, glucose, blood cells, hematocrit rate and hemoglobin of newly hatched male broiler chicks, hatched from eggs injected with ascorbic acid and subjected to heat stress during incubation. For the oxygen saturation of hemoglobin used the portable clinical analyzer (i-STAT ® Co. - Abbott Laboratories - USA), immediately after collection using a cartridge CG8 ® + (i-STAT ® Co. - Abbott Laboratories - USA), using drops of venous blood with anticoagulant heparin. To count the total red blood cell, blood samples were diluted (1:100) and a solution of Natt Henrick (1952). Following this procedure, red blood cell count (n/uL) was performed.

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 blood level data are presented in Table 1. There was no significant effect ($p > 0.05$) of AA and temperature on oxygen saturation in hemoglobin and glucose. For other parameters, erythrocytes (RBC), rate of hematrocit (HCT) and values of hemoglobin (HGB), there was significant interaction ($p < 0.05$) among treatments in egg and incubation temperatures.

Table 1. Effect of injection of ascorbic acid on the RBC, hemoglobin oxygen saturation, glucose, HCT and HGB of newly hatched male broiler chicks.

	Evaluated parameters				
Treatments	RBC**	Hemoglobin oxygen saturation (%)	Glucose (mg/dl)	HCT (%PCU)	HGB *(g/dl)
	ascorbic acid (AA)				
Control	49.18 (3.61)	50.20	156.87	15.36	5.24
0%	58.36 (3.87)	59.00	136.11	14.00	4.75
2%	71.76 (3.55)	46.79	156.50	14.17	4.80
4%	108.42 (3.74)	53.00	149.50	15.67	5.33
6%	67.34 (3.97)	53.00	141.11	13.77	4.68
	Temperatures (TP)				
Termoneutra	69.35 (3.65)	52.14	156.53	14.90	5.06
Quente	63.09 (3.86)	51.23	143.95	14.41	4.90
	Probability for the variance analyses				
AA	0.0006*	0.5482^{NS}	0.3054^{NS}	0.8123^{NS}	0.7972^{NS}
Temperature	<.0001*	0.6821^{NS}	0.1919^{NS}	0.8441^{NS}	0.8703^{NS}
Interaction AA x TP	<.0001*	0.1518^{NS}	0.4487^{NS}	0.0465*	0.0466*
Coefficient of variation (%)	25.23	27.13	18.51	26.15	26.31

^{NS} not significant, * significant at the level 5% of probability. ** comparison from data changed or log (values between parentheses).

With respect to the unfolding of the interaction of RBC (Table 2), broiler chicks from eggs incubated at the hot temperature treatment without injection and with injection of 4 and 6% ascorbic acid, had higher RBC ($p < 0.05$), while for the treatment where 0% ascorbic acid the RBC was higher in broiler chicks from eggs incubated at thermoneutral temperature ($p < 0.05$). These data show that water injection *in ovo* prevented the effect of high temperature incubation. Moreover, these data indicate that the percentage of water present inside the egg influences the effects of temperature on the external development.

Under incubation at thermoneutral temperature, there was a quadratic effect of inoculation levels of AA ($p < 0.05$) on the values of RBC. The maximum rate of the equation ($RBC = 0.0656\ x^2 - 0.4752\ x + 4.1945$ ($R^2 = 0.9714$)) showed that with application of 3.62% AA would have the least amount of RBC. For incubation under hot temperature, there was a cubic effect inoculation levels of ascorbic acid ($p < 0.05$) and the maximum derivative of equation ($RBC = -0.0906\ x\ 3 + 0.765\ x\ 2 - 1.2325\ x\ 3.63 + (R2 = 1)$) estimated that with application of 2.81% AA, would have a lower amount of RBC.

In the comparison between treatment and control application levels of AA, for thermoneutral, only the application of water differed from the control treatment ($p < 0.05$). At the treatment where only water was applied RBC was higher when compared to the control treatment. However, for the hot temperature, application levels of AA did not differ from the control ($p > 0.05$).

Table 2. Unfolding of the interaction to RBC

	RBC [1]		
Treatments	Temperatures (TP)		Probability to the means test
Ascorbic acid (AA)	Thermoneutral	Hot	
Control	45.86 (3.51) b	69.06 (4.16) a	0.0117*
0%	74.19 (4.17) a	45.70 (3.63) b **	0.0007*
2%	99.00 (3.58) **	35.44 (3.50) **	0.6442[NS]
4%	87.35 (3.50) b **	171.63 (3.27) a **	<.0001*
6%	43.88 (3.73) b **	90.81 (4.20) a **	0.0439*
	Probabilidade para os contrastes		
Control vs levels	0.2154 [NS]	0.7964 [NS]	
Linear effect to the levels	0.0301*	<.0001*	
Quadratic effect to the levels	0.0011*	0.0003*	
Cubic effect to the levels	0.4663 [NS]	<.0001*	

[NS]not significant, * significant at 5% probability, a-b comparison in the line. ** Means statistically equal to the control by Tukey test at 5% probability. [1] comparison based on data transformed by log (values in parentheses).

For HCT and HGB (Tables 3 and 4), analyzing the application of AA, it was observed that the treatment with the application of 4% AA *in ovo*, broiler chicks hatched from eggs incubated in hot temperature (39.0 °C) had higher values of HCT and HGB ($p < 0.05$). The increase in HCT has been related to an increased number of blood cells or dehydration. Therefore, this increase in HCT is related to the increase of the values of RBC. The same occurred with the increase of HGB.

According Von Bertalanffy (1960), incubation temperature influences the speed of development, making it faster at higher temperatures. Thus, this increase in embryonic metabolic rate for embryos subjected to heat stress during incubation is the reason for the increase in HCT, and RBC HGB, observed in the present study. Since the increase in metabolic rate in the embryos must have led to an increased number of blood cells, this increase may have been an attempt to supply the necessary oxygen to adapt to the changes of heat production due to the increased metabolic rate.

Tabela 3. Unfolding of the interaction for HCT.

	HCT (%PCU)		
Treatments	Temperatures (TP)		Probability to the means test
Ascorbic Acid (AA)	Thermoneutral	Hot	
Control	17.40	13.67	0.1171 ^{NS}
0%	14.50 **	13.50 **	0.7957 ^{NS}
2%	14.25 **	14.00 **	0.9404 ^{NS}
4%	12.00 b **	18.60 a **	0.0150*
6%	15.33 **	12.43 **	0.1822 ^{NS}
	Probability to the contrasts		
Control vs levels	0.1401 ^{NS}	0.5874 ^{NS}	
Linear effect to the levels	0.9815 ^{NS}	0.8778 ^{NS}	
Quadratic effect to the levels	0.4376 ^{NS}	0.1171 ^{NS}	
Cubic effect to the levels	0.4350 ^{NS}	0.1240 ^{NS}	

not significant, * significant at 5% probability, the line ab comparison. ** Mean statistically equal to the control by Tukey test at 5% probability, compared column.

Tabela 4. Unfolding of the interaction for HGB.

	HGB *(g/dl)		
Treatments	Temperatures (TP)		Probability to the means test
Ascorbic acid (AA)	Thermoneutral	Hot	
Control	5.92	4.67	0.1238 NS
0%	4.90 **	4.60 **	0.8203 NS
2%	4.83 **	4.75 **	0.9477 NS
4%	4.07 b **	6.34 a **	0.0146*
6%	5.21 **	4.21 **	0.1784 NS
	Probabilidade para os contrastes		
Control vs levels	0.1381 NS	0.6093 NS	
Linear effect to the levels	0.9569 NS	0.8878 NS	
Quadratic effect to the levels	0.4423 NS	0.1164 NS	
Cubic effect to the levels	0.4412 NS	0.1177 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.

Conclusion

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

Acknowledgements

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