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EFFECT OF ASCORBIC ACID ON THE ELECTROCARDIOGRAM OF BROILER CHICKENS RAISED AT HIGH ALTITUDE

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SUMMARY

To determine the effect of ascorbic acid on the electrocardiographic parameters of pulmonary hypertension in chickens, broiler chicks were reared from day-old at high altitude and treated with 400, 800 and 1200ppm ascorbic acid for 45 days. Electrocardiograms were recorded at 28, 36 and 45 days of age. RV/TV ratio was noted as an index of pulmonary hypertension. This ratio decreased significantly in 28 (800 and 1200ppm ascorbic acid), 36 and 45 days (all doses of ascorbic acid) compared to controls ($p<0.05$). Decrease at S amplitude in 28, 36 and 45 days by all doses of ascorbic acid (leads II, III, aVL), was only significant in 36 days (lead III, 800 and 1200ppm of ascorbic acid). There were significant reduction of T amplitude in 36 days (lead aVL) and significant elevations of QRS and QT intervals in II lead of 28days at treated groups ($p<0.05$). RR interval also significantly increased just in 28 days (leads aVR, aVL) and 45 days (lead aVL). It was concluded that ascorbic acid modulates induction of pulmonary hypertension, hypertrophy, dilation and arrhythmia of ventricles due to high altitude and these effects are detectable in some of the electrocardiographic parameters, such as T, S amplitudes and QRS, QT, RR intervals.

KEY WORDS: Ascorbic acid, ECG parameters, Mean electrical axis, Altitude, Pulmonary hypertension

INTRODUCTION

Among chickens, meat-producing broiler strains are highly prone to severe pulmonary hypertension and congestive right heart failure. The increased susceptibility of the broiler chicken to pulmonary hypertension is believed to be due to increased metabolic rate and high oxygen requirement causing increased cardiac output, in conjunction with restricted vascular space in the lung that result in pulmonary hypertension (Baghbanzadeh and Decuypere, 2008). However, growth rate, oxygen requirements, organ size and capacity, hematological parameters and cellular responses can all determine how resistant or susceptible a broiler is to pulmonary hypertension

Syndrome. In addition, environmental causes such as altitude, cold stress and rearing condition, such as feed, lighting, air quality and ventilation have all been implicated in pulmonary hypertension development (Balog, 2003). Decreasing the oxygen level to below normal could cause the broiler to become more susceptible to pulmonary hypertension (Jones, 1995) The incidence of pulmonary hypertension has been reported to be greatly increased at altitudes greater than 1300 meters above sea level, presumably because of low oxygen concentration (Hernandez, 1987).

A progressive hypoxia developing in chickens with pulmonary hypertension could be due to increased reactive oxygen species (ROS) by one or more mechanisms. 1) Hypoxia causes increased ROS generation in mitochondria. 2) Tissue damage during hypoxia stimulates white blood cells to infiltrate tissue, which then release ROS causing even more damage (Enkvetchakul, 1994) and 3) Xanthine oxidase activity increases during

hypoxia, which results in high amounts of superoxide being produced (Maxwell *et al.*, 1986).

Ascorbic acid is an important water soluble antioxidant that participates in the elimination of oxygen-derived free radicals (Bottje and Wideman, 1995). It improves endothelium dependent vasodilation in patients with heart failure and hypertension. Ascorbic acid has been suggested to act by enhancing the effects of liberated endothelial vasodilators, and also scavenges reactive oxygen species including superoxide, protects isolated low-density lipoprotein (LDL) against oxidative modification, and plays an important role in the regulation of intracellular redox state (Jacob and Sotoudeh, 2002). A large, prospective population study in human confirmed the evidence for an inverse relation between plasma ascorbic acid concentration and cardiovascular death. This study showed that increasing plasma ascorbic acid concentration was strongly and independently associated with reduction in risk of death from all causes, cardiovascular disease, and ischaemic heart disease (Khaw *et al.*, 2001).

In chickens, ascorbic acid is not routinely added to diets because chickens can synthesize it but under intensive farming conditions and stressors, such as rapid growth, heat, cold, infections, and unsatisfactory diets, chickens may be unable to synthesize adequate amounts of ascorbic acid. Earlier study has shown that ascorbic acid supplementation could reduce mortality among chicks reared under environmental stress (Njoku, 1986). Julian (1992) suggested that ascorbic acid supplementation decreased pulmonary hypertension in T3 treated-chickens, and this reduction of pulmonary hypertension could be a consequence of reduced resistance to blood flow in capillaries of the lungs.

During this study, we used Electrocardiography to investigate the effects of ascorbic acid on pulmonary hypertension syndrome. In previous several studies, electrocardiography has been used to investigate the pathogenesis of some diseases (Odom *et al.*, 1991, 1992; Owen *et al.*, 1995; Wideman and Kirby, 1995a). Changes in electrocardiographic characteristics observed in hypertensive chickens are useful in the diagnosis of the condition even before development of clinical signs (Martinez *et. al.*, 1997; Odom *et. al.*, 1992). The objective of this study was to evaluate the electrocardiographic changes during supplementation by ascorbic acid in broiler chickens at risk of developing pulmonary hypertension due to high altitude.

MATERIALS AND METHODS

Animals, Management and Treatment

A total of 200 day-old Ross 308 breed of broiler chicks were randomly divided into four equal groups (one control and three treatment groups). Chicks were reared in windowless house at standard condition for seven weeks at high altitude (Shahrekord city, Iran; 2100 meters) and provided *ad libitum* access to water and a standard ration (Starter: 3200 kcal metabolizable energy/kg of diet, 23%crude protein; Grower: 3200 kcal metabolizable energy/kg of diet, 20 % crude protein; Finisher: 3200 kcal metabolizable energy/kg of diet, 18% crude protein) formulated to meet requirements of the National Research Council for broilers (National Research Council, 1994). In the treatment groups, ascorbic acid was used from day-old at three doses of 400, 800 and 1200 ppm by dissolving in drinking water; this treated water was accessed *ad libitum.*

Electrocardiographic recordings

At days 28, 36 and 45, 8 chicks from each group were randomly selected and electrocardiograms were recorded by an automatic recorder (Cardiomax FX-2111, Fukuda, Japan) standardized at 10mm =1mv with a chart speed of 50mm/s. Leads I, II , III, aVR , aVL and aVF were recorded for every chicken . Then, the amplitude of T, R, S waves, the intervals of QRS, QT, RR, ST and mean electrical axis (MEA) were measured.

Dissection and assessment of right ventricle hypertrophy

Determination of right ventricle hypertrophy was performed, as previously described by Cuevas *et al.* (1974). The heart was resected, and the atria were removed to the plane of the atrial-ventricular valves, then the total ventricles (TV) were weighed. The right ventricular (RV) wall was then dissected free of the left ventricle (LV) and

septum. The RV was weighed and the RV/TV ratio was calculated.

Statistical analysis

All the data were represented as mean ± SEM. Comparisons were made using unpaired- samples t-test and one-way ANOVA, with $p<0.05$ accepted as significant.

RESULTS

Electrocardiographic parameters and MEA

S, T and R wave amplitudes: There were reductions of the S wave amplitude in 28, 36 and 45 days by three dosages of ascorbic acid (leads II, III, aVL) but were only significant in 36 days (lead III, 800 and 1200 ppm of ascorbic acid) in comparison to controls (Table I). Variations in other leads were not significant. There was only significant decrease of T wave amplitude at 36 days (aVL lead) in treated groups (Table I). Variations in other ages were insignificant. Variations in R wave amplitude were also insignificant. *QRS, QT, RR, ST intervals and MEA:* There were only significant increase of QRS and QT intervals in lead II of 28days at treated groups in the comparison to control (Table II). Variations in other groups were not significant. RR interval also was relatively increased in all ages of treated groups but was just significant in 28 days (leads aVR and aVL) and 45 days (lead aVL) compared to control group. (Table II) (Fig. 1). ST interval and mean electrical axis (MEA) (Table III) did not significantly change in the treated groups in different ages compared to controls, although MEA showed a decrease in most of the treated groups. Electrocardiographic parameters and MEA variations were not statistical significant among treated groups.

Assessment of the right ventricle hypertrophy

The RV/body weight ratios in ascorbic acid-treated groups decreased at different ages and were significant in 28 and 45 days by three doses of ascorbic acid and in 36 days by 800 ppm of ascorbic acid in compared to their control groups (Table III). The RV/TV ratio still decreased in all ages of ascorbic acid-treated groups and were significant in 28 (at doses of 800 and 1200ppm ascorbic acid), 36 and 45 day groups (at all doses of ascorbic acid) when compared to control groups ($p<0.05$) (Table III) (Fig. 1). However, this decrease was moderately 23% in 28days groups, 24% in 36days groups and 22% in 45 days groups. The LV/body weight ratio and TV/body weight ratio decreased in ascorbic acid-treated groups which were not significant (Table III). Statistical analysis showed no significant differences between treated groups in none of the other measured ratios.

TABLE I: Many electrocardiographic wave amplitudes in different groups

		S			T
Age(day)	**Lead Group**	**II**	**III**	**aVF**	**aVL**
	Control	0.25±0.043	0.16±0.014	0.21±0.026	0.08±0.033
	Treat-400	0.24±0.044	0.16±0.033	0.17±0.026	0.07±0.016
	Treat-800	0.21±0.066	0.16±0.041	0.20±0.062	0.07±0.036
28	Treat-1200	0.21±0.042	0.15±0.039	0.18±0.033	0.08±0.009
	Control	0.34±0.062	0.25±0.046	0.29±0.059	0.12±0.013
	Treat-400	0.21±0.062	0.11±0.012*	0.15±0.020	0.03±0.016*
36	Treat-800	0.19±0.047	0.12±0.024*	0.16±0.032	0.05±0.005*
	Treat-1200	0.23±0.044	0.14±0.020*	0.17±0.026	0.07±0.012*
	Control	0.31±0.062	0.18±0.044	0.24±0.036	0.03±0.033
	Treat-400	0.18±0.033	0.13±0.024	0.15±0.027	0.07±0.012
	Treat-800	0.17±0.022	0.11±0.015	0.14±0.021	0.06±0.013
45	Treat-1200	0.18±0.025	0.14±0.021	0.14±0.029	0.06±0.009

*** Significantly different vs. corresponding control ($P<0.05$).**

TABLE II: Cardiac indices and **Mean electrical axis (MEA)** in different groups

		QRS	QT	RR					
Age (day)	Lead Group	II	II	I	II	III	aVF	aVL	aVR
28	Control	0.01±0.003	0.08±0.010	0.08±0.040	0.12±0.003	0.12±0.002	0.12±0.000	0.04±0.042	0.01±0.000
	Treat-400	0.02±0.001*	0.10±0.000*	0.13±0.000	0.13±0.003	0.13±0.003	0.13±0.002	0.13±0.004*	0.13±0.002*
	Treat-800	0.02±0.001*	0.10±0.002*	0.13±0.006	0.13±0.003	0.13±0.002	0.13±0.003	0.13±0.003*	0.13±0.003*
	Treat-1200	0.03±0.002*	0.10±0.002*	0.13±0.002	0.13±0.002	0.13±0.003	0.13±0.002	0.13±0.003*	0.13±0.002*
36	Control	0.02±0.001	0.10±0.005	0.14±0.004	0.13±0.003	0.14±0.003	0.13±0.025	0.13±0.004	0.13±0.006
	Treat-400	0.02±0.000	0.10±0.003	0.14±0.003	0.13±0.003	0.14±0.003	0.13±0.022	0.13±0.044	0.13±0.003
	Treat-800	0.02±0.001	0.10±0.004	0.14±0.004	0.14±0.004	0.14±0.004	0.14±0.005	0.13±0.007	0.14±0.003
	Treat 1200	0.02±0.002	0.10±0.005	0.14±0.003	0.13±0.004	0.14±0.004	0.13±0.002	0.13±0.005	0.13±0.005
45	Control	0.02±0.002	0.10±0.004	0.13±0.005	0.13±0.003	0.13±0.004	0.14±0.005	0.05±0.046	0.13±0.005
	Treat-400	0.02±0.002	0.10±0.004	0.15±0.003	0.15±0.002	0.15±0.003	0.15±0.002	0.15±0.006*	0.15±0.003
	Treat-800	0.02±0.002	0.10±0.003	0.13±0.009	0.14±0.003	0.14±0.003	0.14±0.002	0.13±0.003*	0.19±0.053
	Treat-1200	0.02±0.002	0.10±0.003	0.13±0.003	0.14±0.004	0.14±0.004	0.14±0.004	0.14±0.004*	0.14±0.003

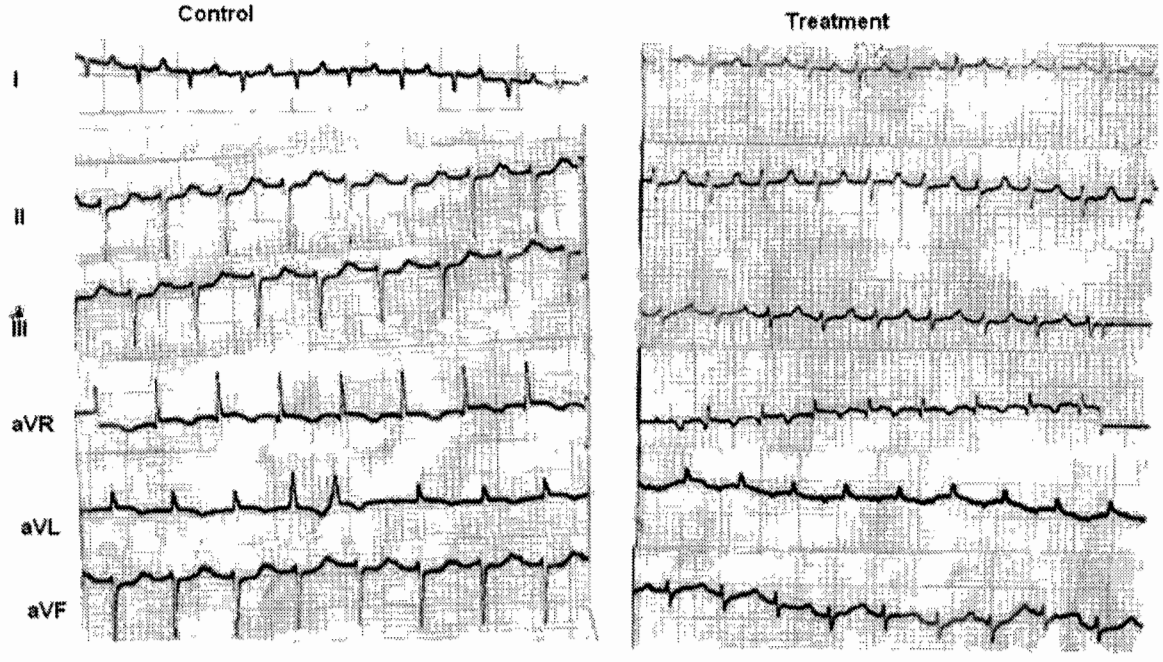

Figure 1: Samples of different electrocardiographs in two groups of hypertensive chickens (control and ascorbic acid-treated). Standardization, 10mm=1mv; chart speed, 50mm/s.

TABLE III: Many electrocardiographic wave intervals in different groups

Age(days)	Group	MEA	RV/TV	%LV/BW	%RV/BW	%TV/BW
	Control	157.28±46.02	0.33±0.01	0.29±0.00	0.14±0.00	0.43±0.01
	Treat-400	254.5±25.37	0.29±0.00	0.27±0.01	0.11±0.00*	0.38±0.02
28	Treat-800	118.37±37.80	0.24±0.01*	0.31±0.00	0.10±0.00*	0.42±0.00
	Treat-1200	201.0±36.49	0.27±0.00*	0.31±0.02	0.11±0.00*	0.42±0.03
	Control	222.50±32.90	0.29±0.02	0.26±0.01	0.11±0.02	0.38±0.03
	Treat-400	112.57±44.1	0.22±0.02*	0.31±0.02	0.09±0.00	0.40±0.02
36	Treat-800	115.37±24.51	0.22±0.01*	0.26±0.01	0.07±0.00*	0.33±0.02
	Treat-1200	152.62±35.78	0.23±0.00*	0.27±0.02	0.08±0.00	0.35±0.03
	Control	173.62±25.99	0.31±0.02	0.24±0.02	0.11±0.00	0.35±0.02
	Treat-400	185.5±33.90	0.24±0.01*	0.24±0.02	0.08±0.00*	0.32±0.01
45	Treat-800	204.25±43.66	0.25±0.00*	0.25±0.02	0.08±0.01*	0.34±0.03
	Treat-1200	141.62±35.46	0.25±0.00*	0.24±0.00	0.08±0.00*	0.32±0.01

*** Significantly different vs. corresponding control (P <0.05).**

DISCUSSION

In this study, we found that ascorbic acid can reduce induction of pulmonary hypertension due to high altitude since RV/TV ratio was lower in the treated groups than the control, and lower than 0.28 which is the level considered for pulmonary hypertension syndrome (Wideman, 2001). These results agreed with the findings of Julian (1992). Ladmakhi *et al.* (1997) also showed that dietary T3 supplementation increased pulmonary hypertension and mortality. Addition of ascorbic acid to the feed reduced this mortality. Xiang *et al.* (2002) also showed that ascorbic acid reduced pulmonary hypertension and the associated muscularisation of pulmonary arterioles induced by exposing broilers to cool environmental temperatures and feeding them with T3.

Previous studies by Hassanpour *et al.* (2005) on electrocardiographic parameters of cold-induced pulmonary hypertension in broilers showed that T and S waves had significant elevations which also agreed with the reports of Odom *et al.* (1991), Owen *et al.* (1995), Wideman and Kirby (1995; 1996) and Martinez *et al.* (1997). Their results could also be used as evidence that dilation and hypertrophy of ventricles were the primary cause of the increased amplitude of S wave (long ventricle depolarization) (Hassanpour *et al.*, 2005). In the present study, it was shown that T and S waves decreased during administration of ascorbic acid at various ages. Therefore it was suggested that broilers supplemented by ascorbic acid had lower rate of hypertrophy and dilation of ventricles. QRS, QT and RR intervals were also increased at different ages of chickens signifying an evidence for inhibition of tachycardia in ascorbic acid-supplemented chickens with pulmonary hypertension (Schamroth, 1985; Cinar *et al.*, 2006).

CONCLUSION

It was concluded that supplementation of the ascorbic acid can modulate induction of pulmonary hypertension, hypertrophy, dilation and arrhythmia of ventricles due to high altitude and these effects are detectable in some electrocardiographic parameters, such as T, S wave amplitudes and QRS, QT, RR intervals.

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