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Effects of stress produced by adrenocorticotropin (ACTH) on ECG and some blood parameters in vitamin C treated and non-treated chickens

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ABSTRACT

The aim of this study was to examine the effects of vitamin C on ECG and some blood parameters in chickens stressed by ACTH. Sixty Leghorn chickens were randomly allotted to control and experimental groups. Prior to the experimental period ECG's were recorded and blood samples were taken from both groups of chickens; some blood parameters were also determined. In the control group, isotonic sodium chloride solution was administered at a dose of 2.5 mL per chicken per day for a period of 5 days. In the experimental group, vitamin C (Redoxon Amp, Roche) was administered intramuscularly at the same dose (containing 250 mg vitamin C) and period of time. On the 5th day of the experiment chickens in both groups received 50 IU ACTH intramuscularly. Three hours following this application ECGs were recorded and blood samples were taken from both groups of chickens; some blood parameters were also determined. In conclusion, sinusoidal tachycardia was observed and an increase was determined ($P<0.05$) in the level of corticosterone and heterophil/lymphocytes (H/L) ratio after ACTH application in the control group.

Key words: adrenocorticotropin, blood parameters, electrocardiography, vitamin C

Introduction

Stress affects many systems and causes a decrease in animal production. Many researchers (SIEGEL, 1971; STEVENSON and TAYLOR, 1988; BELGE et al., 2003) have reported that stress can be produced experimentally by ACTH. Stress is believed to cause

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many diseases in the living organism due to its negative effects, which are as follows: depression of immune system against infections, reduction in rate of reproduction and growth in the living bird, spoiling many metabolic functions, causing feeding disorders, etc. (DAVISON et al., 1988; GRAY et al., 1989; KODAMA et al., 1994; PUVADOLPIROD and THAXTON, 2000). These disorders may result in individual or mass deaths in poultry. Part of these deaths is the result of heart diseases that can be diagnosed by ECG and blood parameters (ACKERMAN and HAMLIN, 1976; GROSS, 1966; PATTERSON and SIEGEL, 1998). Thereby, the effects of many diseases and environmental conditions on ECG and some blood parameters have been studied (STURKIE et al., 1954; YERSIN and EDENS, 1987; ÇINAR and DONMEZ, 2001).

Although studies with ECG date back to 1948 (STURKIE, 1948), the number of studies on ECG in poultry has been very limited in recent years. Due to the thickness of poultry chest muscle it is impossible to obtain any recording by using chest leads. Therefore, ECGs of poultry are recorded and evaluated by using limb leads (ÇINAR et al., 1996). The ECG recording in poultry was obtained using bipolar and augmented unipolar limb leads by STURKIE (1949). Since amplitude of lead I is low or iso-electric, lead II is usually obtained from poultry in order to reduce errors (GROSS, 1966; ÇINAR et al., 1996). Mean electrical axis can also be found in lead II and lead III (STURKIE, 1949; ÇINAR et al., 1996).

Ascorbic acid is not typically added to poultry diets because chickens can synthesize it (ascorbic acid, vitamin C), (SATTERLEE et al., 1989). However, the metabolic need for ascorbic acid is likely to exceed in innately pathological situations and stresses. Therefore, vitamin C is added to poultry diets together with other vitamins (YERSIN and EDENS, 1987; McKEE and HARRISON, 1995; BASPINAR et al., 1998; ANDREASEN and FRANK, 1999; PUTHPONGSIRIPORN et al., 2001; SAHIN et al., 2002). Stress is generally associated with a decline in production performance. The suppression of adrenocortical steroidogenesis by ascorbic acid may constitute the primary reason that this vitamin can ameliorate the negative effects of stress (PARDUE, 1987; SATTERLEE et al., 1989).

Although many researchers have investigated the effects of stress and vitamin C on some blood parameters (McKEE and HARRISON, 1995; PUVADOLPIROD and THAXTON, 2000; BELGE et al., 2003), the effects of vitamin C on ECG have not yet been adequately investigated. Therefore, it would be useful to establish the effects of vitamin C on ECG and some blood parameters in chickens stressed by ACTH.

Materials and methods

Sixty 20-week-old Leghorn chickens were used in this study. The chickens were divided into two groups: control and experimental. Prior to the experimental period ECGs of the chickens were recorded and then blood samples were collected from both groups to determine some blood parameters. While isotonic sodium chloride solution was

administered intramuscularly to the control group at a dose of 2.5 mL per chicken per day for a period of 5 days, vitamin C (Redoxon Amp, Roche) was administered intramuscularly to the experimental group at the same dose (containing 250 mg vitamin C) and period of time (BELGE et al., 2003). Food and water were given ad libitum to both groups during experiment. On the 5th day of the experiment, chickens in both groups received 50 IU ACTH intramuscularly (Synackten Depot, Ciba) (TROUT and MASHALY, 1994; BELGE et al., 2003). ECGs of chickens were recorded and blood samples were taken to determine some blood parameters after three hours following ACTH application.

Alligator clip electrodes were attached to the skin at the base of the right and left wings and gastrocnemius muscle of the right and left limbs. Electrode gel was applied to the skin in the area where the alligator clips were attached to act as decreasing agents that decrease the resistance of the skin (STURKIE, 1948). The chickens were immobilized by wrapping with a light cotton fabric (STURKIE, 1949; ÇINAR and DÖNMEZ, 2001), rested on a table and left for about 10 minutes to calm down. The chickens were not anesthetized at any time. All recordings were obtained on the same day.

Electrocardiograms were recorded by a direct writing electrocardiograph (Cardiofax 6851, Nihon Kohden Tokyo). All ECGs of the chickens were standardized at 1 milivolt (mV) = 10 mm, with a chart speed of 50 mm per second (STURKIE, 1948; ÇINAR et al., 1996). Leads I, II, III, aVR, aVL and aVF were recorded (STURKIE, 1949; ÇINAR et al., 1996). The durations and amplitudes of waves on the trace were measured in lead II (STURKIE, 1948; ACKERMAN and HAMLIN, 1976; ÇINAR et al., 1996), and electrical axis was also measured in leads II and III (STURKIE, 1949; SULU et al., 1993; ÇINAR et al., 1996). Blood samples were analyzed according to KONUK (1981). Plasma corticosterone was determined by RIA. All data were subjected to analysis of variance using General Linear Model procedure of SAS. Mean treatment differences were determined by Duncan's multiple range test with a level of statistical significance of 5% (STELL and TORRIE, 1980).

Results

All leads of the electrocardiogram of the Leghorn chickens are seen in Figures 1 and 2.These readings represent the majority of the Leghorn chickens. The durations and amplitudes of all waves in lead II and some blood parameters are shown in Table 1.

In lead I, traces were either iso-electric or were of very low amplitude. The P wave was PT form (P and T waves were together) and the Q wave was not found in the chickens' ECG. The S wave was higher than R wave in the majority of leads. The T wave in leads I, II, III and, aVF was observed positive and negative in leads aVR and aVL (Figs. 1, 2). Although the heart rate was 300 beats/min before and 431 beats/min after the experiment in the control group, it was 305 beats/min before and 317 beats/min after the experiment in the experimental group. Sinusoidal tachycardia was observed after ACTH application

in control group. The heart rate was higher in the control group than in the experimental group three hours after ACTH application (P<0.05) (Table 1). The average values of the mean electrical axis of heart were-70^0 and-78^0 before and-77^0 and-80^0 after experiment

Table 1. Heart rate, ECG values and some parameters in vitamin C-treated and control group chickens

	Control group (2.5 mL isotonic NaCl)		Experimental group (250 mg Vit C in 2.5 mL)	
ECG values	Before the experiment	After the experiment	Before the experiment	After the experiment
Heart rate	300 ± 39[a]	431 ± 37[b]	305 ± 33[a]	317 ± 51[a]
QRS (sec.)	0.05 ± 0.007 [a]	0.03 ± 0.006[b]	0.05 ± 0.007[a]	0.05 ± 0.0068[a]
QRS (mV)	0.5 ± 0.09	0.6 ± 0.08	0.48 ± 0.08	0.5 ± 0.09
T (P+T) (sec.)	0.16 ± 0.072[a]	0.12 ± 0.068[b]	0.16 ± 0.08[a]	0.15 ± 0.073[a]
T (mV)	0.2 ± 0.07	0.3 ± 0.08	0.21 ± 0.08	0.2 ± 0.09
Electrical axis	-70^0 ± 15	-78^0 ± 12	-77^0 ± 11	-80^0 ± 14
White blood cell (10^3/mm^3)	33 ± 8[a]	38 ± 6[a]	34.5 ± 6[a]	42 ± 6[b]
H/L	0.39 [a]	2.6 [b]	0.42 [a]	1.04 [a]
Corticosterone (ng/mL)	1.25 ± 0.29 [a]	13.0 ± 4.0 [b]	1.22 ± 0.19	1.48 ± 0.07

[a,b] Means in the same row with different superscripts differ significantly (P<0.05)

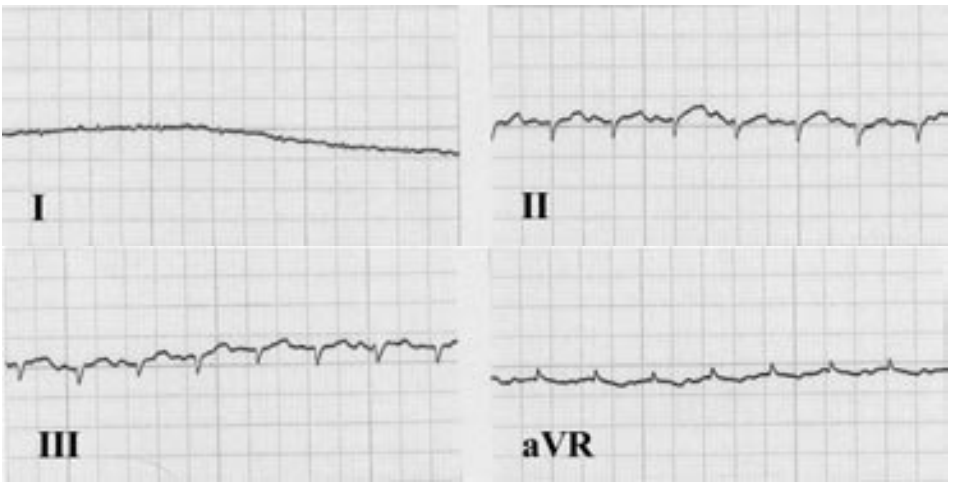

Fig. 1. Electrocardiogram of the healthy Leghorn chicks (standardization, 1 mill volt = 10 mm; chart speed, 50 mm per second).

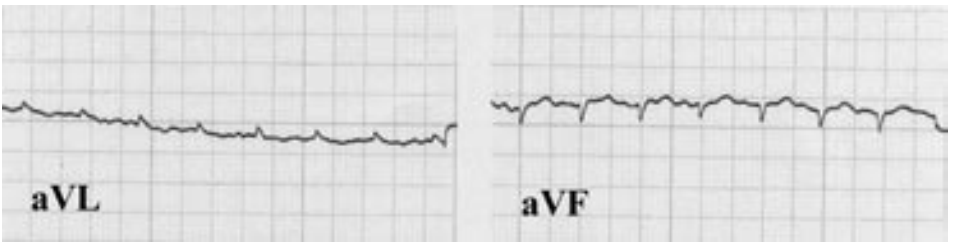

Fig. 1. (continued) Electrocardiogram of the healthy Leghorn chicks (standardization, 1 mill volt = 10 mm; chart speed, 50 mm per second)

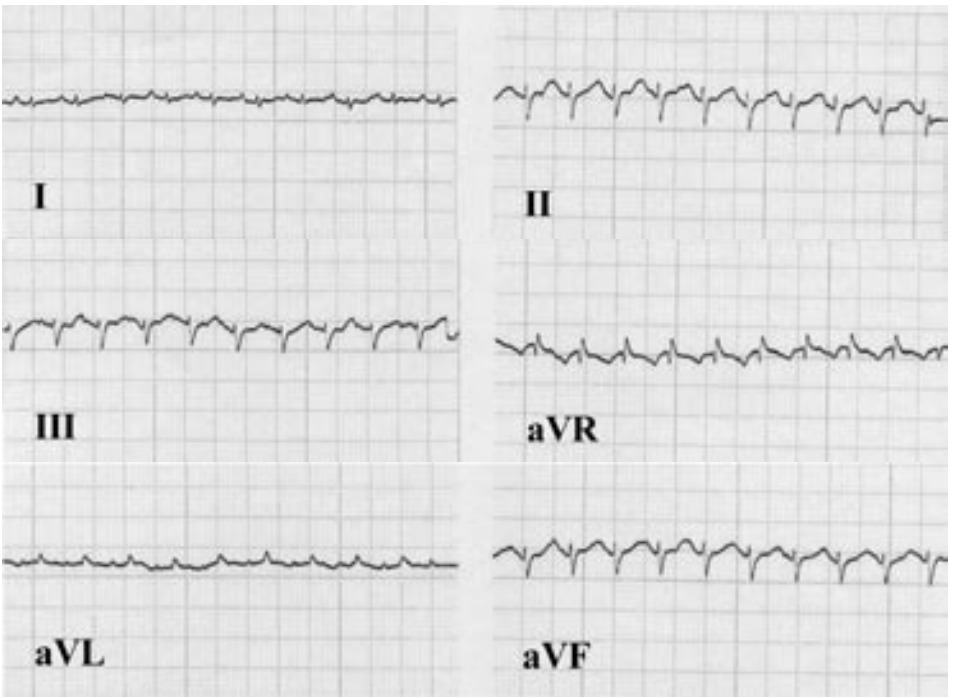

Fig. 2. Sinusoidal tachycardia in control Leghorn chickens stressed by ACTH (standardization, 1 mill volt = 10 mm; chart speed, 50 mm per second)

(range-60^0 and-110^0) in both control and experimental groups, respectively. ACTH caused an increase in plasma corticosterone (CS) and heterophil/lymphocytes (H/L) ratio.

Discussion

The P wave was seen generally in PT form. Before and after experiment the mean duration of QRS complex that represents ventricular depolarization was different in the groups (0.03 ± 0.006 and 0.05 ± 0.007 sec) and the mean amplitude of QRS complex changed between 0.5 ± 0.09 and 0.6 ± 0.08 mV. The duration and amplitude of the QRS complex were higher than the values reported in the literatures (STURKIE, 1948; 1949; ÇINAR et al., 1996). The duration of QRS complex was shorter in control than in treatment group due to the presence of sinusoidal tachycardia. The S wave was usually greater than the R wave (rS) in all leads.

The T wave was positive in all leads except in leads aVR, and aVL. These findings were also consistent with the information given in the literature (STURKIE, 1948; 1949; ÇINAR et al., 1996; ÇINAR and DÖNMEZ, 2001). There were negative correlations between duration of T wave and heart rate. The amplitude of T wave changed between 0.2 and 0.3 mV in this study. Although these values are similar to the values given in the literatures (SULU et al., 1993; ÇINAR et al., 1996), they were higher than the results (0.109 mV) given by STURKIE (1948; 1949) and lower than the results given by STURKIE et al. (1954). There were positive correlations between the amplitude of T wave and QRS complex (ÇINAR et al., 1996; ÇINAR and DÖNMEZ, 2001), although T wave can be positive, negative or iso-electrical (STURKIE, 1948; 1949; ÇINAR et al., 1996). The duration of T wave shortened after ACTH application in the control group ($P<0.05$).

Mean heart rates before and after the experiment in control and experimental groups were 300 ± 39, 431 ± 37 beats/minute and 305 ± 33, 317 ± 51, respectively. These findings were similar to the literature findings (STURKIE et al., 1954; ÇINAR et al., 1996; ÇINAR and DÖNMEZ, 2001). Increase in heart rate response to stress was observed in both groups. However, this increase was statistically significant ($P<0.05$) in the control group, but not in the experimental group (Table 1). Sinusoidal tachycardia observed in the control group following ACTH application might be due to the effect of stress (Table 1). The level of corticosterone increased after application of ACTH in the control group, but not in the experimental group. Ascorbic acid may functionally inhibit ACTH-mediated steroidogenesis by altering cell membrane lipid composition/structure and thus in uencing ACTH binding to its membrane-bound receptor. Ascorbic acid may also inhibit enzymes of the adrenal steroidogenic pathway, which inhibits the conversion cholesterol to pregnenolone, causing the decreased levels of corticosterone in blood. This may be a result of the response to the acute stress of ascorbic acid in which glicocorticoid and mineralocorticoid are decreased.

These results revealed that vitamin C adjusts the organism against stress and protects it against negative the effects of the stress.

In this study, however, white blood cells increased in the vitamin C-treated group ($P<0.05$) and slightly increased in the stressed control group. This increase was not statistically significant. The number of white blood cells has been reported to be unchanged (PATTERSON and SIEGEL, 1998), decreased (SIEGEL, 1971), and increased (GRAY et al., 1989) in stress by different researchers. This can be explained by a decrease as a result of leucopenia (SIEGEL, 1971), and an increase due to a rise in heterophil rate (GRAY et al., 1989). However, it may be a result of both the decreases in vessel permeability which limits leucocytes infiltration and the increase in the passing of the heterophils from the bone marrow to blood circulation. Increase in white blood cells in this study was in consistent with the reported data (GRAY et al, 1989; ANDREASEN and FRANK, 1999). Decrease in the rate of lymphocyte (ANDREASEN and FRANK, 1999; PUVADOLPIROD and THAXTON, 2000), increase in the rate of heterophils (BASPINAR et al., 1998) and the rise in the rate of H/L were parallel to present findings obtained under different stress conditions in chickens (DAVISON et al., 1988; STEVENSON and TAYLOR, 1988; ANDREASEN and FRANK, 1999; PUTHPONGSIRIPORN et al., 2001). Increase in the rate of heterophil was the most sensitive haemopoietic sign of chronic stress. Decrease in the circulating lymphocytes may be the result of cell destruction or the redistribution of cells to those out of the circulation. It is clear that vitamin C provides adaptation to stress by protecting the immune system from the negative effects of corticosteroids.

ACTH may inhibit with vitamin C, and decreases the release of glicocorticoids and mineralocorticoids in the adrenal gland, and norepinephrine from sympathetic nerve ends. Stress hormones arrange somatomotor and visceromotor activities according to body needs. The response for stress-changes according to dosage and time of the factor and physiologic adaptation which produces stress. In conclusion, vitamin C may improve immunity, facilitate the adaptation response to against stress and decrease negative effects on production in chickens.

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SAŽETAK

Cilj rada bio je istražiti učinke vitamina C na ECG i neke pokazatelje u krvi pilića u kojih je izazvan stres s ACTH. Šezdeset pilića leghorn pasmine razvrstano je metodom slučajnog izbora u pokusnu i kontrolnu skupinu. Prije pokusa u obje skupine pilića višekratno je mjeren ECG te uzimani uzorci krvi sa svrhom određivanja nekih pokazatelja u krvi. Pilićima u kontrolnoj skupini davana je izotonična otopina natrijeva klorida u količini 2,5 mL po piletu u tijeku 5 dana. Pilićima pokusne skupine u istom je razdoblju i u istoj količini (5 dana; 250 mg po piletu) dan vitamin C (Redoxon Amp, Roche). Petoga dana pokusa pilići obiju skupina dobili su 50 IJ ACTH intramuskularno. Tri sata nakon primjene ACTH u pilića obje skupine višekratno je mjeren EKG-a i uzimani su uzorci krvi sa svrhom određivanja nekih pokazatelja u krvi. Zaključno, u pilića kontrolne skupine nakon primjene ACTH utvrđena je sinusoidalna tahikardija te povećana razina kortikosterona ($P<0,05$) i promijenjeni omjer heterofila i limfocita (H/L).

Ključne riječi: adrenokortikotropin, pokazatelji u krvi, elektrokardiografija, vitamin C