Effects of the level of copper supplementation in diet on electrocardiogram of Japanese quails (Coturnix coturnix japonica)

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SUMMARY

The aim of this study was to assess the effects of various levels of copper supplementation on electrocardiogram (ECG) of Japanese quails (Coturnix coturnix japonica). For that purpose, 75 chicks (one-day-old) were divided into four groups and they received 0 (Group I), 100 (Group II), 250 (Group III) and 500 (Group IV) mg/kg copper in addition to their concentrate for 6 weeks. Each group were subdivided into females and males. Fresh water and feed was offered ad libitum and all ECGs were standardized at 1 mV = 20 mm, with chart speed of 50 mm / s. Leads I, II, III, aVR, aVL and aVF were determined. Heart rate was not different between the groups (P>0.05) but it was insignificantly lower in females (range 464-513 beats/min) than in males (range 504-521 beats/min). Mean electrical axis, calculated from leads II and III, did not differ between the groups and it was between -76° and -89° in females, and between -83° and -91° in males. Waves in lead I had the lowest amplitudes and they were isoelectric. P wave was detected only in some of the leads (III, aVR, aVL) and it was generally fused into the T wave. T wave was negative in aVR and aVL leads whereas it was positive in leads I, II, III, aVF. Q wave was, however, observed only in aVL lead. QRS complex was in the form of QS or Rs. There were no differences between the groups or sexes in amplitude and duration of QRS complex and in duration of T (P-T), P-Q or Q-T. In conclusion, it was observed that Japanese quail chicks are able to tolerate, in terms of ECG findings, diets containing copper at a level as high as 500 ppm.

Keywords : Japanese quail - copper - electrocardiogram - diet.

RÉSUMÉ

Effets du niveau de la supplémentation en cuivre dans l’aliment sur l’électrocardiogramme des cailles japonaises (Coturnix coturnix japonica). Par F. ONDER*, M. CENESIZ, M. KAYA, M. UZUN et S. YILDIZ.

Le but de cette étude était d’évaluer les effets de divers niveaux de supplémentation en cuivre sur l’électrocardiographe (ECG) des cailles japonaises (Coturnix coturnix japonica). Dans ce but, 75 poussins (âgés d’un jour) ont été divisés en quatre groupes et ont reçu 0 (groupe I), 100 (groupe II), 250 (groupe III) et 500 (groupe IV) mg/kg de cuivre dans leur concentré pendant 6 semaines. Chaque groupe a été subdivisé en femelles et mâles. L’eau et l’alimentation étaient fournies ad libitum et tous les ECGs ont été normalisés à 1 mv = 20 mm, avec une vitesse de papier de 50 mm/s. Les dérivations II, III, aVR, aVL et l’aVF ont été étudiées. La fréquence cardiaque n’était pas différente entre les groupes (P>0.05) mais elle était inférieure chez les femelles (de 464 à 513 battements par min) par rapport aux mâles (504 à 521 battements par min) sans que la différence ne soit significative. L’axe électrique moyen, calculé à partir des dérivations II et III, n’a pas différé entre les groupes et il était compris entre -76° et -89° chez les femelles, et entre -83° et -91° chez les mâles. Les ondes dans la dérivation I présentaient les amplitudes les plus faibles et étaient isoelectriques. L’onde P a été détectée seulement dans certaines dérivations (III, aVR, aVL) et elle a été généralement superposée avec l’onde T. L’onde T était négative dans les dérivations aVR et aVL mais elle était positive dans les dérivations I, II, III, aVF. On a cependant observé une onde Q seulement en aVL. Le complexe QRS était sous la forme QS ou Rs. Il n’y avait aucune différence entre les groupes ou les sexes dans l’amplitude et la durée du complexe QRS et dans la durée de T (P-T), de P-Q ou de Q-T. En conclusion, il est apparu que les poussins japonais de cailles pouvaient tolérer, en termes d’effets électrocardiographiques, d’ECG, des régimes contenant du cuivre à un niveau aussi élevé que 500 ppm.

Mots-clés: Caille japonaise - cuivre - électrocardiogramme - aliment.

Introductions

Copper is one of the essential trace elements and is incorporated in several vital enzymes, such as Cu, Zn-SOD, dopamine β-hydroxylase, ceruloplasmin, cytochrome c oxidase etc. [22]. Both its deficiency and excess lead to physiological disturbances and diseases [12]. During the deficiency phases, abnormalities on cardiac function have been reported [6, 23]. It has been observed that copper deficiency increases the Q-T interval in the electrocardiogram (ECG) of pigs [31] and rats [6, 23] with also an increasing amplitude of QRS in the latter [6, 23]. In fact, copper poisoning or supra normal copper level is not uncommon as it is used as fungicide [24], as a treatment regimen for foot diseases [18] and also as an industrial substance (mining) [8]. Copper is a component of the normal ration in animals and therefore mistakes in calcula-

lation might result in poisoning [14]. Similarly, in some pathological conditions, blood copper concentrations raises to a supra normal level [32] and it is also raised in some neurodegenerative diseases [21]. Additionally, cardiac abnormalities have been suggested to be correlated closely with increased copper status [27]. ECG gives significant information about the details of cardiac activity and serves as a laboratory aid in diagnosis of cardiovascular diseases [2]. This technique has widely been used for the diagnosis of variety of diseases in chickens [10, 17] or for the assessment of the effect of various drugs in pigeon [28]. The data reported in the previous studies, thus, provide the rationale to assess the effects of high copper intake on cardiac function.

There is ample evidence of the copper necessity for normal cardiac function but effects of excess copper on cardiac func-

tion is unclear. Therefore, the aim of this study is to investigate the effects of copper on ECG findings in Japanese quail chicks (Coturnix coturnix japonica) fed with diets possessing various levels of copper (0, 100, 250, 500 mg/kg feed) for 6 weeks.

Materials and methods

1. ANIMALS AND EXPERIMENTAL DESIGN

In this experiment, a total of 75 (36 females, 39 males) one-day-old Japanese quail chicks were divided into four groups as follows: Group I, 10 females and 9 males; Group II, 8 females and 10 males; Group III, 10 females and 11 males; Group IV, 8 females and 9 males. For 6 weeks, chicks in Group I (Control) were offered a concentrate containing 2900 kcal/kg metabolisable energy and 22 % crude protein. The other groups were fed with the same concentrate supplemented by 100, 250 or 500 mg/kg Cu as copper sulphate pentahydrate (CuSO₄·5H₂O). Feed and water were provided ad libitum. At the end of the study period, ECGs were recorded.

2. ECG RECORDINGS

ECGs were recorded in a quite room and quails were calmed down by covering a cloth on them for 5 min before recording [29]. Thus, movements and artefacts were minimised. The ECGs were recorded by a direct writing electrocardiograph (Logos 8821, Logos Medical Co. Ltd., Tokyo, Japan). Alligator clip electrodes were attached to the skin at the base of right and left wings and gastrocnemius muscle of the right and left limbs. All ECGs were standardised at 1 mV = 20 mm, with chart speed of 50 mm/s. Leads I, II, III, aVR, aVL and aVF were determined. The duration and amplitude of waves on the trace were measured in lead II and mean electrical axis was calculated using leads II and III. Heart rate was calculated from lead II. [4].

3. STATISTICAL ANALYSIS

Data were analysed using Generalised Linear Models (GLM) within MINITAB statistical package (MINITAB Inc., PA, USA). Analysis included diet, sex, and their interactions. When significance was found in GLM, data were analysed by Student’s t-test to find out where the difference had occurred. The differences were considered as significant when p values were less than 0.05. Data are presented as mean ± Standard error (S.E.M).

Results

ECG findings of quail chicks are displayed in Table I and traces of ECG in male Japanese quails that received 500 mg/kg copper are presented in Figure 1.

Although heart rates were slightly lower in females (464-513 beats/min) than in males (504-521 beats/min), the difference was not statistically significant. Amplitude of waves in lead I was very small and isoelectrical. Although P wave was only observed in leads III, aVR and aVL, it was generally fused into the T wave. Q wave was only observed in lead aVL. QRS complex was in the form of QS or rS. T wave was in the form of P-T, and was positive in leads I, II, III and aVF and negative in leads aVR and aVL.

Among the parameters acquired, there was no difference among groups and between female and male except that the amplitude of T (P-T) wave in the males of Group II was higher compared to the T wave of the other groups (P< 0.05) (Table I).

Discussion

This study shows that copper intake by Japanese quails for 6 weeks has no detrimental effect upon cardiac parameters as measured by ECG. Various assumptions might be done: Firstly, the source of copper used in the current study, i.e. copper sulphate pentahydrate, is also the recommended supplement for poultry. It is normally added to broiler and laying hens diets at prophylactic dosages due to its antimicrobial and growth promoting effects [1, 7]. Secondly, the dose used is appropriate for testing our hypothesis as DOZIER et al. [7] recommended 4-12 mg/kg copper sulphate for broiler chicks. Therefore, any argumentation about insufficiency of copper in the current study might be ruled out. Experimental period was also of sufficient duration as MILES et al. [15] observed very significant Cu amounts in the liver of chicks within 21 days of supplementation with 450 mg/kg copper sulphate. In fact, by increasing Cu doses from 0, 150, 300 to 450 mg/kg in the diet, they observed linear increases of the liver Cu amounts irrespective of source of Cu [15]. Therefore, in the current experiment, the doses, duration of application and type of copper seemed to be suitable for testing the effects of copper on ECG. Taken together, data in the current study suggest that, in terms of ECG findings, copper has no deleterious effects upon the function of the heart. Similarly, in an in situ study, 1-100 μmol copper application to heart of sharks had no effect on heart rate and ECG findings [30]. Probably, heart is not the site of negative action of excess copper. Rather, liver and kidneys seem to be major accumulation sites [15, 16].

P wave was transposed into the T wave as it was also observed for other avian species [5, 9] (Figure 1). The Q wave was observed only in lead aVL (Figure 1) but CINAR et al. [4] observed it on both leads aVR and aVL in ducks. However, the Q wave was either not observed in poultry [9, 26] and falcons [20] or was observed in lead aVR in turkeys [11]. The QRS complex, i.e. ventricular depolarisation, was in the form of QS or rS (Figure 1) as reported by others for other avian species [13, 20]. The duration of QRS wave was 0.021-0.028 s (Table I), which was found to be similar to the values obtained for chickens [5, 9] and parrots [3] but lower than those of pekin ducks [4] and falcons [20]. Amplitude of the QRS wave was also similar to those of hens [25] and falcons [20], but was lower than those of parrots [3]. Although it has been reported that copper deficiency decreases the amplitude of QRS complexes in rats [6, 23], the extra copper supplementation resulted in no changes of the QRS complex in the current study.
ONDER (F.) AND COLLABORATORS

**TABLE I.** The durations and amplitudes of waves in lead II, mean electrical axis and heart rate in the Japanese quails receiving different level of copper in feed.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sex</th>
<th>QRS (QS,rs) (sec)</th>
<th>QRS (QS,rs) (mV)</th>
<th>T(T-P) (sec)</th>
<th>T(T-P) (mV)</th>
<th>(P-T) - Q (R) (sec)</th>
<th>Q-T(R)-(P-T) (sec)</th>
<th>Heart rate (beats/min)</th>
<th>Electrical axis (°)</th>
</tr>
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<tbody>
<tr>
<td><strong>Group I</strong></td>
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<tr>
<td>(0 mg/kg Cu)</td>
<td>Female (n=10)</td>
<td>0.021 ± 0.002</td>
<td>0.13 ± 0.02</td>
<td>0.053 ± 0.003</td>
<td>0.09 ± 0.01*</td>
<td>0.050 ± 0.004</td>
<td>0.080 ± 0.002</td>
<td>468 ± 17</td>
<td>- 79 ± 7</td>
</tr>
<tr>
<td></td>
<td>Male (n=9)</td>
<td>0.027 ± 0.002</td>
<td>0.16 ± 0.01</td>
<td>0.051 ± 0.004</td>
<td>0.12 ± 0.01*</td>
<td>0.040 ± 0.004</td>
<td>0.090 ± 0.004</td>
<td>504 ± 21</td>
<td>- 88 ± 6</td>
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<tr>
<td><strong>Group II</strong></td>
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<tr>
<td>(100 mg/kg Cu)</td>
<td>Female (n=8)</td>
<td>0.028 ± 0.002</td>
<td>0.16 ± 0.03</td>
<td>0.051 ± 0.004</td>
<td>0.10 ± 0.01*</td>
<td>0.030 ± 0.003</td>
<td>0.180 ± 0.089</td>
<td>513 ± 27</td>
<td>- 80 ± 6</td>
</tr>
<tr>
<td></td>
<td>Male (n=10)</td>
<td>0.026 ± 0.002</td>
<td>0.15 ± 0.03</td>
<td>0.050 ± 0.003</td>
<td>0.23 ± 0.06*</td>
<td>0.040 ± 0.005</td>
<td>0.090 ± 0.004</td>
<td>509 ± 24</td>
<td>- 83 ± 6</td>
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<tr>
<td><strong>Group III</strong></td>
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<tr>
<td>(250 mg/kg Cu)</td>
<td>Female (n=10)</td>
<td>0.024 ± 0.002</td>
<td>0.18 ± 0.01</td>
<td>0.048 ± 0.002</td>
<td>0.10 ± 0.01*</td>
<td>0.030 ± 0.005</td>
<td>0.090 ± 0.003</td>
<td>502 ± 24</td>
<td>- 89 ± 4</td>
</tr>
<tr>
<td></td>
<td>Male (n=11)</td>
<td>0.026 ± 0.002</td>
<td>0.20 ± 0.02</td>
<td>0.046 ± 0.002</td>
<td>0.12 ± 0.01*</td>
<td>0.040 ± 0.005</td>
<td>0.080 ± 0.002</td>
<td>521 ± 23</td>
<td>- 91 ± 6</td>
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<tr>
<td><strong>Group IV</strong></td>
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<tr>
<td>(500 mg/kg Cu)</td>
<td>Female (n=8)</td>
<td>0.024 ± 0.002</td>
<td>0.15 ± 0.02</td>
<td>0.049 ± 0.003</td>
<td>0.10 ± 0.02*</td>
<td>0.040 ± 0.005</td>
<td>0.080 ± 0.003</td>
<td>464 ± 25</td>
<td>- 76 ± 5</td>
</tr>
<tr>
<td></td>
<td>Male (n=9)</td>
<td>0.023 ± 0.003</td>
<td>0.18 ± 0.02</td>
<td>0.042 ± 0.001</td>
<td>0.09 ± 0.009*</td>
<td>0.030 ± 0.001</td>
<td>0.080 ± 0.003</td>
<td>505 ± 18</td>
<td>- 89 ± 5</td>
</tr>
</tbody>
</table>

(Different superscripts within a column differ significantly at p< 0.05).

**FIGURE 1.** The electrocardiogram of the female Japanese quails that dietary received 500 mg/kg copper for 6 weeks (standardization, 1 mV=20 mm; chart speed, 50 mm/sec).
The T wave was in the form of P-T and was positive in leads I, II, III and aVF but negative in leads aVR and aVL (Figure 1) as reported by others [11, 26]. Heart rates seemed to be slightly lower in female quails than in males (but not significantly). Similarly, PEARSON et al. [19] observed higher heart rates in male quails. This fact appears to be related to the lower body weight in females [4]. Above all, heart rates observed in the current study were higher than those of hens [5, 9, 26], ducks [4], falcons [20], parrots [3] and partridges [29].

Electrical axis of the quails in the current study (-76° and -91°) was similar to the values obtained from turkeys [2], broilers [5] and chickens [26] but was lower than that of macaws (-81° and -131°) [3]. From the data in the current study, it is possible to postulate that the heart is situated frontally slightly towards left hand side.

In conclusion, the data in the current study suggest that copper supplementation of the diets (as high as 500 ppm) of Japanese quails for 6 weeks has no detrimental effects upon electrocardiographical findings.

References