Effects of dietary CrCl$_3$ supplementation on some serum biochemical markers in broilers. Influence of season, age and sex

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SUMMARY

The effects of season, age, sex and dietary Cr supplementation on some serum biochemical markers were investigated in broilers. A total of 600 Ross-PM3 one day old broiler chicks (300 in winter and 300 in summer) were used. In each experimental period, 150 male and 150 female chicks were divided into two groups according to dietary CrCl$_3$ supplementation (0 ppm in control, 20 ppm CrCl$_3$.6H$_2$O in treated group). Blood samples were collected on the 1$^{st}$, 3$^{rd}$ and 6$^{th}$ weeks of age and the serum AST, CK activities, inorganic phosphorus (iP), K, total protein, albumin, globulin, and Cr concentrations and body weight were determined. Season greatly influenced all analysed markers (p<0.01) except serum K concentrations. Significant differences between male and female chicks were observed for serum AST activity (p<0.001), globulin concentration (p<0.05) and body weight (p<0.001). All markers were significantly changed with aging (p<0.001). Dietary Cr supplementation significantly increased AST and CK activities (p<0.05), mineral concentrations (p<0.001) and body weight (p<0.001). In conclusion, although season, age, sex and Cr treatment affected blood markers, they stayed within normal ranges. Besides, because improvement of body weight gain, dietary Cr supplementation (20 ppm) may be economically beneficial in broilers.

Keywords : Biochemical markers - Broiler - Chromium - Body weight.

RéSUMÉ

Effets d’une supplémentation alimentaire en CrCl$_3$ sur différents marqueurs biochimiques sériques chez le poulet. Influence de la saison, de l’âge et du sexe. Par M. EREN et N. BASPINAR.

Les effets de la saison, de l’âge, du sexe et d’une supplémentation alimentaire en Cr sur plusieurs marqueurs biochimiques sériques ont été recherchés chez les poulets de chair. Au total 600 poussins Ross PM3 âgés de 1 jour (300 en hiver et 300 en été) ont été utilisés. Pour chaque saison, 150 mâles et 150 femelles ont été répartis en 2 groupes de taille égale selon l’apport supplémentaire en Cr : 0 ppm dans le groupe contrôle et 20 ppm de CrCl$_3$.6H$_2$O dans le groupe traité. Les animaux ont été pesés et les prélèvements sanguins ont été effectués à la 1$^{re}$, 3$^{ème}$ et 6$^{ème}$ semaines afin de mesurer les activités enzymatiques sériques en AST et CK, les concentrations sériques de phosphates inorganiques (iP), K, protéines totales, albu- mine et globulines. La saison a grandement influencé tous les marqueurs étudiés (p<0.01) à l’exception de la kaliémie. L’activité sérique en AST (p<0.001), la concentration en globulines (p<0.05) et le poids corporel (p<0.001) ont présenté des différences significatives entre mâles et femelles. Tous les marqueurs testés ont été significativement modifiés par l’âge (p<0.001). La supplémentation alimentaire en Cr a significativement augmenté les activités en AST et CK (p<0.05), les concentrations des miné- raux (p<0.001) et le poids corporel (p<0.001). En conclusion, bien que la saison, l’âge, le sexe et le traitement en Cr aient affecté les marqueurs sanguins étudiés, ces derniers sont restés compris dans les valeurs normales. Comme en plus, le gain de poids a été amélioré, une supplémentation alimentaire en Cr (20 ppm) peut se révéler économiquement rentable chez le poulet.

Mots-clés : Marqueurs biochimiques - poulet - chrome - poids corporel.

Introduction

Chromium (Cr$^{3+}$) is an essential element required for the metabolism of carbohydrates, proteins and lipids in animals and man [28]. Chromium affects structural integrity of nucleic acids, stimulates the action of insulin [3], and improves immunity [6, 16]. Chromium picolinate (CrPic) affected serum inorganic phosphorus (iP) concentrations in pigs [18], but high Cr-yeast had no effect on serum potassium (K) concentration in calves [16]. Chromium had no effect on serum alkaline phosphatase (ALP) in pigs [18] and rabbits [26], whereas this element increased serum ALP in calves [6]. However, there is no study related the effects of Cr on serum aspartate amino transferase (AST) and creatine kinase (CK) enzymes.

The effects of chromium on protein metabolism [5, 6, 12, 13, 15] and on body weight [5, 6, 12, 16, 21, 25, 27] are dependent of the animal species and of the dietary Cr chemical form. In poultry, studies about potential Cr supplementation effects on serum total protein, albumin [12], globulin concentrations and body weight [25] are scarce.

This experiment was performed to determine the effects of inorganic Cr on serum AST and CK activities, on iP, K, total protein, albumin, globulin concentrations and on body weight in male and female broilers during summer and winter seasons.

Material and Methods

A total of 600 Ross-PM3 broiler chicks consisting of 300 chicks in summer and 300 chicks in winter were used. In each experimental period, 150 male and 150 female chicks...
were divided into two groups as control (fed with basal diet) and treatment groups (fed with basal diet + 20 mg/kg Cr as CrCl\(_3\cdot6\)H\(_2\)O). The chicks were intensive bred and housed as 75 animals/pen on wire-separated-floor pens in a room on a 24-h constant lighting schedule. The room temperature was set to 35°C for the first week of age and then gradually reduced to 28°C with thermostatically controlled electrical heaters. Animals were fed with starter diets (3 000 kcal/kg, 22% crude proteins) during 30 days then with grower diets (3 000 kcal/kg, 20% crude proteins) until the 42\textsuperscript{nd} day. Ingredients and chemical composition of basal diet fed to broilers was shown in Table I. Feed and water was supplied ad libitum.

Blood samples were collected at the 1\textsuperscript{st}, 3\textsuperscript{rd} and 6\textsuperscript{th} weeks of age. Sera were separated by centrifugation at 1300g for 15 min following a 1-h incubation at room temperature and stored at -20°C until analysis of biochemical markers. Serum AST and CK activities, iP (Biocon, Germany), total protein, albumin (Valtek, Chile) concentrations were analysed by an AE-100F Erma Inc. Microflow-Cell Photometer using commercial kits. The serum globulin concentrations were calculated by subtracting the albumin values from total protein values [8]. Serum K concentrations were determined with Hitachi Model 2-8000 Polarize Zeeman atomic absorption spectrophotometer and Cr content of basal diet was determined with same equipment with HGA-600 graphite furnace [19]. Also, serum Cr concentrations were determined with Model Zeeman/5100 PC atomic absorption spectrophotometer with HGA-600 graphite furnace [20].

Statistical analysis was performed by SPSS 6.0 version for Microsoft. Multifactorial variance analysis was used [23]. When the F values were significant, Duncan’s Multiple Range Test was performed. Results are considered as significant when p values were less than 0.05. All data were expressed as means ± SEM.

Results

Table II presented the influence of different parameters (season, sex, age and dietary Cr supplementation) on the analysed markers whereas serum enzyme activities, mineral concentrations and body weight for each subgroup were reported in Table III. Statistical interactions between parameters were reported in Table IV.

SEASON INFLUENCE

Serum enzyme AST and CK activities and iP concentrations increased in winter (p<0.001), whereas serum total protein, albumin, globulin concentrations (p<0.001), Cr concentrations (p<0.01) and body weight (p<0.001) decreased (Table II). However K concentrations were not influenced by season.

SEX INFLUENCE

Serum AST activity was significantly increased in females (p<0.001) whereas globulin concentrations (p<0.05) and body weight (p<0.001) were significantly higher in males. Sex as a mean factor did not modify the other markers (Table II).

AGE INFLUENCE

For all tested markers, significant fluctuations due to aging were observed. Firstly, body weight markedly increased and was maximal at the 6\textsuperscript{th} week (p<0.001). Serum protein concentrations were modified in the same way: the highest total serum protein, albumin and globulin concentrations (p<0.001) and body weight (p<0.001) decreased (Table II). However K concentrations were not influenced by season.

Table 1: Ingredients and chemical composition of basal diets fed to broilers.

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>Starter (1-30 day)</th>
<th>Grower (20-42 day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>27.13</td>
<td>50.00</td>
</tr>
<tr>
<td>Full fat soya</td>
<td>27.7</td>
<td></td>
</tr>
<tr>
<td>Soy meal</td>
<td>46.00</td>
<td>27.7</td>
</tr>
<tr>
<td>Sunflower meal</td>
<td>8.62</td>
<td></td>
</tr>
<tr>
<td>Cottonseed meal</td>
<td>11.43</td>
<td>5.00</td>
</tr>
<tr>
<td>Meat-bone meal</td>
<td>6.53</td>
<td>4.00</td>
</tr>
<tr>
<td>Fish oil</td>
<td>3.55</td>
<td></td>
</tr>
<tr>
<td>Fish meal</td>
<td>3.27</td>
<td></td>
</tr>
<tr>
<td>Sorghum</td>
<td>2.70</td>
<td></td>
</tr>
<tr>
<td>Lime stone</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td>Vitamin-Mineral mix</td>
<td>0.56</td>
<td>0.25**</td>
</tr>
<tr>
<td>Salt</td>
<td>0.32</td>
<td>0.20</td>
</tr>
<tr>
<td>Antibacterial-anticoagdial</td>
<td>0.16</td>
<td>0.20</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.24</td>
<td>0.05</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td><strong>Cr (μg/g)</strong></td>
<td>0.6147</td>
<td>0.7533</td>
</tr>
</tbody>
</table>

*: Provided by per kg of diet: vitamin A, 15 000 IU, vitamin D3, 3000 IU, vitamin E, 20 mg, vitamin K3, 5 mg, vitamin B1, 2.5 mg, vitamin B2, 7.5 mg, vitamin B6, 5 mg, vitamin B12, 0.020 mg, folic acid, 0.75 mg, calcium pantothenate, 10 mg, ascorbic acid, 50 mg, monensin sodium, 100 mg, cholin chloride, 400 mg, nicotinamide, 25 mg, D-biotin, 0.05 mg, manganese, 80 mg, iron, 40 mg, zinc, 60 mg, copper, 5 mg, iodine, 0.4 mg, selenium, 0.15 mg, cobalt, 0.1 mg, antioxidant, 10 mg.

**: Provided by per kg of diet: vitamin A, 10 000 IU, vitamin D3, 1200 IU, vitamin E, 20 mg, vitamin K3, 3 mg, vitamin B1, 2 mg, vitamin B2, 6 mg, vitamin B6, 4 mg, vitamin B12, 0.015 mg, folic acid, 0.35 mg, calcium pantothenate, 5 mg, monensin sodium, 100 mg, cholin chloride, 500 mg, nicotinamide, 25 mg, manganese, 80 mg, iron, 40 mg, zinc, 60 mg, copper, 5 mg, iodine, 0.4 mg, selenium, 0.15 mg, cobalt, 0.1 mg, antioxidant, 10 mg.

Table 1: Ingredients and chemical composition of basal diets fed to broilers.
EFFECTS OF DIETARY CrCl₃ SUPPLEMENTATION ON BIOCHEMICAL MARKERS IN BROILERS

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INFLUENCE OF DIETARY Cr TREATMENT

Significant increases of Cr concentrations in groups fed with Cr enriched diets (p<0.001) evidenced the efficiency of dietary Cr supplementation. Cr treatment has induced significant increases of electrolyte (iP, K) concentrations (p<0.001), of enzyme activities (AST: p<0.01, CK: p<0.05) and of body weight (p<0.001), whereas serum protein concentrations (total protein, albumin and globulin) were comparable in control and in treated groups.

INTERACTIONS BETWEEN PARAMETERS: SEASON, AGE, SEX AND CR SUPPLEMENTATION

No interaction between these parameters was noticed for CK activity and globulin concentration (Table 4). The variations of AST activity due to aging were amplified in females (p<0.05), and the effect of Cr supplementation was more important in older broilers (p<0.001). Season greatly influenced variations of AST activity: in winter, the fluctuations due to age (p<0.001) or to Cr supplementation (p<0.001) were enhanced. Moreover, the changes in AST activity in

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**Table 2.** Variations of AST and CK activities, iP, K, total protein (TP), albumin (Alb), globulin (Glob) and Cr concentrations and body weight (BW) in broilers according to season, sex, age and dietary Cr supplementation. Results are expressed as means ± SEM. abc: The mean values within the same lines with different superscript differ significantly. *: p<0.5; **: p<0.01; ***: p<0.001; -: not significant.

**Table 3.** Variations of AST, CK activities, iP, K, total protein (TP), albumin (Alb), globulin (Glob), Cr concentrations and body weight (BW) in each subgroup of broilers according to season, sex, age and dietary Cr supplementation. Results are expressed as means ± SEM. abc: The mean values within the same column with different superscript differ significantly. ***: p<0.001, ND: not detectable.

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females according to age were more marked in winter. By contrast, the increases of serum total protein concentrations in treated females were more relevant during summer (p<0.05) and the aging fluctuations were more marked (p<0.05). The variations of albumin concentrations according to age were enhanced in females (p<0.05) and in Cr-treated broilers (p<0.001). Season/age interactions have been observed for serum mineral concentrations (iP: p<0.01, K and Cr: p<0.001): the decreases of mineral concentrations according to age were more pronounced in summer for iP and in winter for K and Cr. For this latter, females in winter exhibited strong aging concentration changes (p<0.05). Season as interaction factor significantly affected iP concentrations: in winter, the increases of iP concentrations were more evident in males (p<0.001) or in treated birds (p<0.01), particularly in younger treated chicks (p<0.001). Furthermore, a significant season/age/sex/Cr treatment interaction was observed for iP concentrations (p<0.05): they were amplified in treated males (p<0.01) and especially in younger treated males in winter (p<0.001). In summer, high K concentrations were obtained in males (p<0.001) or in Cr supplemented birds (p<0.001), particularly in males (p<0.05) or in younger treated chicks (p<0.001). Chromium supplementation amplified age related K variations (p<0.05), especially in males (p<0.05). The age-induced increases of body weight were exacerbated in summer (p<0.001) and in males (p<0.001). Besides, the body weight gain was greater in Cr treated birds (p<0.001).

### Discussion - Conclusion

Blood constituents can be influenced by many biological factors (such as genetic type, age, sex, infection, management, climate and other stress factors) and also by technical factors (sampling protocols and methods of analysis) [4, 14].

It has been reported that Cr absorption was depressed in old humans [7]. Similarly, serum Cr concentrations decreased with age in this study. Serum AST activities were comparable with previous reports and changed with aging, with a drop of enzyme activity in 21 day old chicks following by recovery at 42-45 days [4, 14]. Serum CK activities obtained in our study were in good agreement with ITOH’S and UYANIK’S studies [10, 26] which have also observed enzyme activity fluctuations with aging. Gradual increases of serum total protein, albumin and globulin concentrations were evidenced according to the age of broilers. The obtained values were similar to previous reports [2, 4, 14] although higher globulin concentrations were noticed in 13 month old rooster [22]. Blood mineral concentrations found in our study were decreased in older birds and were in accordance with previously reported values [4, 14]. Age influence has already been reported on iP concentrations [14] and on K concentrations [24], but BOWES et al [4] did not obtain significant variations of iP and K concentrations according to age.

Marked increases of enzyme (AST, CK) activities and of Cr and iP concentrations were found in winter. MELUZZI et al [14] have also reported season influence and they have evidenced increases of AST activity and iP concentrations in summer contrary to our results. These discrepancies would be due to different climate, management and nutrition conditions. Greatest serum Cr, total protein, albumin and globulin concentrations were obtained in summer. These findings supported previous reports [14] which also observed higher total protein and albumin concentrations in summer.

Females presented significant greater serum AST activities than males as previously indicated by SREEMANNaRAyANA et al [24], whereas sex did not influence CK activity. Although differences of globulin concentrations were noticed between male and females, serum albumin and total protein concentrations were identical in both sexes. These results were in agreement with previous reports [14, 24]. On the contrary, BOWES et al [4] have observed lower albumin and total protein concentrations in males than in females. As in BOWES’s study [4], no difference between males and

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**Table 4.**—Statistical interactions evidenced between the different parameters tested (season, sex, age, Cr treatment) for each biochemical markers and for body weight.

<table>
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<tr>
<th>Source of Variance</th>
<th>AST</th>
<th>CK</th>
<th>iP</th>
<th>K</th>
<th>TP</th>
<th>Alb</th>
<th>Glob</th>
<th>Cr</th>
<th>BW</th>
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<td>Season x age</td>
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*: p<0.5; **: p<0.01; ***: p<0.001; -: not significant
females has been observed for iP concentrations. By contrast, MELUZZI et al [24] reported that sex influenced electrolyte concentrations in broiler. No sex effect was evidenced for K concentrations in our study. Contrary to that, SREEMANNARAYANA et al [24] reported lower values in males. Nevertheless, it was probable that Cr supplementation affected K concentrations and occulted the sex differences. For Cr concentrations, no significant difference occurred between males and females as in ABRAHAM’s study conducted in humans [1].

Dietary Cr supplementation induced significant increases of Cr concentrations in broilers in agreement with previous reports [11] and of mineral concentrations (iP and K). However PAGE et al [18] failed to obtain changes of iP concentrations when birds were supplemented with Cr Picolinate. These discrepancies would result from the different nature of administrated Cr. Marked increases of AST and CK activities occurred in dietary Cr supplemented birds, indicating that Cr could accelerate liver and muscle metabolisms. Serum total protein, albumin and globulin concentrations were not modified by Cr supplementation in our study. Similarly, these biochemical markers were not affected by Cr supplementation neither as Cr Picolinate in lambs [13], in steers [5] or in pigs [15] nor as CrCl3 in pigs [15].

In this study, dietary Cr supplementation significantly affected body weight. Similar increases were obtained in rat [21] and in turkey [25] fed with inorganic Cr enriched diets, in calves received high Cr-yeast and chelated Cr [6, 16] and in pigs treated by Cr Picolinate [27]. The improvement of body weight by Cr supplementation would result from increases of nucleic acid synthesis and amino-acids uptake [9, 17].

In conclusion, the analysed serum biochemical markers stayed within normal ranges although season, age, sex and Cr supplementation diversely affected them. Besides, as body weight gain was improved in CrCl3 treated birds, dietary Cr supplementation (20 ppm) may be economically beneficial.

References

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