The Effect of Dietary Chromium Supplementation on Blood Biochemical Parameters of Broiler Chicks

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This study investigated the effect of dietary supplementation of chromium from chromium picolinate source on the blood biochemical parameters of broilers. A three hundred sixty-one day old commercial male broiler were randomly allocated to one of 24 floor pens in a completely randomized design with six treatments and four replicate groups and fifteen chicks per each pen. Dietary treatments consisted of zero (control), 400, 800, 1200, 1600 and 2000 µg kg⁻¹ Cr of basal diets. At 42 days of age, two birds from each pen were selected and blood samples were collected from wing vein. Concentrations of high density lipoprotein (HDL), low density lipoprotein (LDL), glucose, cholesterol, triglyceride, total protein and insulin were measured. There were not significant effects of supplementary Cr on HDL and glucose concentrations. But favourably cholesterol (P = 0.0002), triglyceride (P = 0.0004), total protein (P = 0.0003), insulin (P = 0.0004) and LDL (P = 0.058) significantly affected by Cr supplementation. Birds receiving Cr supplementation had lower LDL, cholesterol, triglyceride and higher total protein and insulin concentrations. It can be concluded that under conditions of this experiment Cr addition to broiler diets have an effect on lipid metabolism in broilers.
INTRODUCTION

Chromium (Cr) is a metallic element with poor absorption, its been used as a marker for the passage of foods and nutrients through the gastrointestinal tract (McDowell, 1992). Broiler diets contain large proportion of plant products which are low in Cr content demonstrating potentiality of Cr deficiency in broilers (Schroeder, 1971; Gibson, 1989). Reported values for feedstuffs range from 0.01 to 4.2 mg kg\(^{-1}\) DM Cr, with cereals relatively poor and legumes relatively rich in Cr (Underwood and Suttle, 1999).

Presence of Cr in the glucose tolerance factor (GTF) molecule caused Cr to be recognized as a GTF, which enhances metabolism of Glucose, intensifies glycogenesis from glucose and speeds up glucose transport (Rosebrough and Steele, 1981). The organic compound GTF is more active biologically (about 50 times) than inorganic Cr trivalent (Cr\(^{3+}\)) (McDowell, 1992). Desirable absorption, different tissue distribution, and availability to the embryo are denominated for organically bound (GTF) form of the Cr (Mertz and Roginski, 1971).

Established data reveals that Cr hexavalent is inorganic and toxic, also it has poor absorption (0.5-3.0 %), instead trivalent Cr is organic form with 25-30% bioavailability (Mowat, 1994). Cr is a necessary trace element in animal body (NRC, 1980), and its essentiality for mammals was first proved by Schwartz and Mertz (1959), who demonstrated enhancements in glucose tolerance by means of supplements of trivalent Cr in rats.

Regarding metabolism numerous studies have validated prominent function of Cr in the metabolism of carbohydrates, lipids, proteins and nucleic acids (Steele and Rosebrough, 1981; Okada et al., 1984; Anderson and Kozlovsky, 1985; McCarty, 1991), although potentiating the function of insulin via organometallic GTF molecule is the original function of Cr in the metabolism (Anderson, 1987; Sahin et al., 2001; Pechova et al., 2002; Sahin et al., 2003).

Enhancement in lipogenesis from glucose and lipid storage into liver and adipose tissues are associated with Cr functions (Steele and Rosebrough, 1979). Cr can increases high-density lipoproteins (HDL) and reduce lipid, low-density lipoproteins (LDL), total cholesterol (Press et al., 1990). Reduced plasma cholesterol (Anderson, 1986; Press et al., 1990; Boelman et al., 1995; Lien et al., 1998, 2001) and egg yolk cholesterol contents (Lien et al., 1996) are some other mentioned effects of CrPic supplementation. Present study investigates the effects of various dietary concentrations of CrPic on blood biochemical parameters of broiler chicks.

MATERIALS AND METHODS

Animals and Diets and its Experimental Design

A three hundred sixty- one day old commercial male broilers (Ross 308) were randomly allocated to one of 24 floor pens in a single brooder house with six treatments and four replicate groups and fifteen chicks per each pen.

The dietary treatments consisted of the supplementation of the basal diet with 0 (control), 400, 800, 1200, 1600, 2000 mg kg\(^{-1}\) Cr in diet), supplied from chromium picolinate (CrPic: Assay 99.20% and Cr content 12.30%). CrPic was first mixed with specific amount of mineral premix and then blended with small amounts of basal diet, afterward larger amounts of basal diet were mixed until a homogeneous mixture of the diet was obtained. Experimental diets were provided ad libitum. All pens were equipped with feeders and waterers. The birds were fed by either a control diet or the control diet supplemented with Cr until the day of 21st as starter, followed by a finishing diet from the day of 21st to the day of 42nd. The ingredients and chemical composition of the starter and grower basal diets are shown in Table 1. The basal diets were prepared based on corn-soybean meal and formulated according to NRC (1994) guideline, contained 20.48-18.25% crude protein (CP) and 2850-2920 kcal kg\(^{-1}\) apparent metabolizable energy (AME).
Table 1: Composition of the basal diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Starter (1-21)</th>
<th>Grower (22-42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn grain</td>
<td>62.04</td>
<td>68.32</td>
</tr>
<tr>
<td>Soybean Meal (44% CP)</td>
<td>34.33</td>
<td>28.15</td>
</tr>
<tr>
<td>Di Calcium Phosphate</td>
<td>1.42</td>
<td>1.2</td>
</tr>
<tr>
<td>Oyster Shell</td>
<td>1.25</td>
<td>1.44</td>
</tr>
<tr>
<td>Common Salt</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Vitamin Premix¹</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Mineral Premix²</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.14</td>
<td>0.1</td>
</tr>
<tr>
<td>L-Lysin mono hydro chloride</td>
<td>0.02</td>
<td>0.0</td>
</tr>
</tbody>
</table>

**Calculated Analysis**

<table>
<thead>
<tr>
<th>Metabolizable Energy (ME) (kcal kg⁻¹)</th>
<th>2850</th>
<th>2920</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP (%)</td>
<td>20.48</td>
<td>18.25</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Available Phosphorus (%)</td>
<td>0.40</td>
<td>0.35</td>
</tr>
<tr>
<td>Sodium (%)</td>
<td>0.13</td>
<td>0.14</td>
</tr>
<tr>
<td>Chloride (%)</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>Potassium (%)</td>
<td>0.87</td>
<td>0.77</td>
</tr>
<tr>
<td>Chromium³ (mg/kg)</td>
<td>0.041</td>
<td>0.036</td>
</tr>
<tr>
<td>Chromium content of drinking water³ (ppm)</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Methionine + Cysteine (%)</td>
<td>0.80</td>
<td>0.70</td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>1.10</td>
<td>0.935</td>
</tr>
<tr>
<td>Tryptophan (%)</td>
<td>0.29</td>
<td>0.25</td>
</tr>
</tbody>
</table>

¹- Vitamin Premix provides the following per kg: Vit. A, 4800000 IU; Vit. D, 1000000 IU; Vit. E, 10000 IU; Vit. K, 850 mg; thiamin (B1), 605 mg; riboflavine (B2), 2400 mg; panthothenic acid (B3), 3990 mg; nicotinic acid (B5), 13930 mg; Pyridoxine hydrochloride (B6), 121 mg; folic acid (B9), 1190 mg; cyanocobalamin (B12), 8 mg; biotin (H2), 80 mg; choline chloride, 120000 mg.

²- Mineral premix provides the following per kg: manganese, 46.8 mg; iron, 14.8 mg; zinc, 27 mg; copper, 2.475 mg; iodine, 0.44 mg; selenium, 0.06 mg.

³- Chromium content of the basal diet and drinking water was determined by atomic absorption spectrophotometer.

Sample Collection and Laboratory Analysis

After 6 weeks on the experimental diets followed by 12 hour of fasting, two chicks per each pen (eight birds per each treatment) were chosen based on weight closeness to the mean of each pen as representative sample and two blood samples were taken from the wing vein of each bird and the blood was centrifuged at 5000 rpm for 15 minutes to obtain serum and then stored at -20°C. The concentrations of following criteria were measured with a commercial kit package (Pars Azmoon) and an autoanalyzer (MINDRAY BS-200 Chemistry Analyzer): HDL, LDL, glucose, cholesterol, triglyceride, and total protein. Also insulin was assayed using an enzymatic immunoassay kit package (Monobind, Germany).

Analyses of Chromium in Foods and Water

Concentrations of Cr in the starter and the finisher foods also in water was determined by atomic absorption spectrometer with a graphite furnace (VARIAN spectrAA 220) according to the methodology suggested by the Perkin-Elmer (1982) with some modifications. The samples of ground foods (5 g) were oven-dried and then de-carbonized in a glazed ceramic crucible at 180 °C. The samples were then ignited in a muffle furnace at 400 °C for 4 h. The ash (0.5 g) was treated with concentrated nitric acid under mild heat to oxidize the trivalent forms of Cr to the hexavalent (Cr VI) form, which could be detected more accurately. After complete digestion, the acid-extracted sample was cooled at room temperature and filtered through ash-lees Whatman filter paper (No. 1). The crucible was washed several times with triple distilled water and the final volume was made up to 10 ml. Subsequent analysis of Cr was done in the atomic absorption spectrophotometer. A 2% solution of ammonium chloride was added in both the standard and experimental samples to reduce interference caused by the presence of Fe in the samples.

Statistical Analyses

General linear model of SAS statistical program (SAS, 1997) was used to identify the variance among groups. Significant differences between treatments means were examined with Duncan's new multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Table 2 summarizes the effect of dietary supplementation of Cr on blood biochemical parameters of broilers at the end of experiment. According to the obtained results, it was observed that adding Cr supplementation to the broiler diet had no significant effect on blood glucose. However, numerically treatments containing Cr had lower blood glucose. Lee et al. (2003) did not observe changes in blood glucose concentration in chicks fed 200 to 800 µg kg⁻¹ using CrPic form. Sands and Smith (2002) also reported that supplementation of diet with CrPic did not affect blood glucose of broilers reared under natural or heat stress conditions. Kim et al. (1996) also used CrPic at 800 µg kg⁻¹ of diet and did not observe changes in blood glucose levels of broilers but...
reported reduced glucose concentration in blood serum at 1600 and 2400 µg kg\(^{-1}\). Lien et al. (1999) observed no changes in serum glucose at 800 µg kg\(^{-1}\) of diet, besides reported decreases in serum glucose while using 1600 and 3200 µg kg\(^{-1}\). Reduction in blood glucose concentrations can be attributed to the role of insulin in uptake of glucose. In this regard, results of this study showed that effect of Cr supplementation on insulin hormone was significant (\(P = 0.0004\)).

Treatments mean showed that, with increasing amounts of dietary Cr, serum insulin levels were significantly increased. It has been shown that the main roles of Cr are increasing concentrations of insulin and reduction in blood glucose concentration, increasing the number of insulin receptors which make insulin physiologically active (Anderson et al., 1991). Insulin stimulates anabolism and prevents catabolism, which result in increased uptake of blood glucose and consumption of glucose by the cells (Rosebrough and Steele, 1981; Cupo and Donaldson, 1987). Insulin also inhibits gluconeogenesis and reduces adipocyte lipolysis by reducing the activities of adenylate cy clase and hormone-sensitive lipase (Lambert and Jacqumin, 1979). Interactions described above, resulting in decreased concentrations of glucose and non-esterified fatty acids concentrations. In particular, insulin can increase the lipoprotein lipase activity and eventually decrease the contents of TG-rich lipoproteins (VLDL) (Garfinkel et al., 1976; Howard et al., 1993). The results of this experiment are in agreement with those of researchers listed above where concentrations of triglyceride (\(P=0.0004\)), LDL (\(P=0.05\)) and cholesterol (\(P=0.0002\)) influenced by dietary supplementation with Cr but HDL (\(P=0.1922\)) concentration was not affected by Cr supplementation.

### Table 2: Effect of dietary supplemental chromium on biochemical parameters of broilers at different period of ages

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Treatments (chromium levels) µg kg(^{-1})</th>
<th>SEM(^1)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (g mL(^{-1}))</td>
<td>173.18, 165.25, 168.12</td>
<td>160.18, 169.87, 167.50</td>
<td>6.87</td>
</tr>
<tr>
<td>Insulin (pmol L(^{-1}))</td>
<td>85.43(^{abc}), 145.62(^{a})</td>
<td>67.50(^{c})</td>
<td>129.37(^{a})</td>
</tr>
<tr>
<td>Triglyceride (mg dL(^{-1}))</td>
<td>90.87(^{a}), 78.18(^{a})</td>
<td>79.75(^{a})</td>
<td>55.37(^{a})</td>
</tr>
<tr>
<td>LDL (mg dL(^{-1}))</td>
<td>35(^{a}), 33.87(^{ab})</td>
<td>37.56(^{a})</td>
<td>33.56(^{a})</td>
</tr>
<tr>
<td>HDL (mg dL(^{-1}))</td>
<td>22.87, 22.61</td>
<td>22.06, 23.31</td>
<td>23.41, 25.41</td>
</tr>
<tr>
<td>Cholesterol (mg dL(^{-1}))</td>
<td>105.68(^{a}), 99.06(^{a})</td>
<td>103.50(^{a})</td>
<td>99.06(^{a})</td>
</tr>
<tr>
<td>Total protein (g dL(^{-1}))</td>
<td>6.22(^{a}), 6.85(^{a})</td>
<td>6.49(^{a})</td>
<td>7.60(^{a})</td>
</tr>
</tbody>
</table>

\(^{abc}\) Means in the same row with no common superscript are significantly different (\(P<0.05\))

\(^{1}\)SEM: Standard Error of Mean

Lien et al. (1999) did not observe effect of CrPic on serum cholesterol and HDL levels of broiler chickens. Sands and Smith (2002) also did not report positive effect of Cr supplementation on concentrations of cholesterol, LDL and HDL-cholesterol in blood serum of chickens reared in normal and heat stress conditions. Kroliczewska et al. (2004) observed reduction in serum triglyceride concentrations of broiler chicks fed with organic Cr supplementation; on the contrary, Lien et al. (1999) reported no change and Chen et al. (2001) observed increases in serum triglyceride concentration of broilers and turkeys with use of Cr supplantations.

Serum total protein concentration were significantly affected by dietary supplementation with Cr (\(P=0.0003\)). In this regard, the control group had lowest and the 1200 µg kg\(^{-1}\) treatment had highest concentration of total protein. Sahin et al. (2002) comparably reported similar result. Al-Bandr et al. (2010) also reported significantly increased plasma total protein of broilers while using Cr-yeast and CrPic. Kroliczewska et al. (2004), on the contrary, reported that supplementation of broiler diet with different levels of Cr-yeast did not influence blood serum protein concentration at 21 and 42 days of age. Increase in total serum protein can be justified by the role of Cr on insulin function as a factor in the absorption of amino acids in animal tissues, although its mechanism is still not clearly defined.

In conclusion, the results of this study reveal that supplementation of CrPic in broiler diet have an effect on lipid metabolism and improve some serum criteria.

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### REFERENCES


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