Influence of Zinc and Chromium Supplementation on Blood Biochemical Constituents and Hematological Profiles in Goats

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors HK and SPT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HK, BK and SK managed the analyses of the study. All authors read and approved the final manuscript.

ABSTRACT

Aims: An experiment was conducted to investigate the effect of supplementing zinc-methionine and chromium-picolinate on blood biochemical constituents and hematological profiles of goats.

Place and Duration of Study: The present studies were carried out at Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Anjora, Durg during February 2007 to August 2007.

Methods: Twenty-four indigenous non-descript goat kids (3-5 months old, average body weight of 5.1±0.25 Kg) were allocated to four groups Group-I (control) was fed ad libitum basal complete feed which constituted sola (Aeschynomene indica) - hay (60 parts) and concentrate mixture (40 parts). The kids of Group-II, III and IV were fed as in control with a combined supplement of Zn-methionine and Cr-picolinate.
and Cr-picolinate at the rates of 20 and 0.5 parts per million (ppm), 35 and 1 ppm and 50 and 1.5 ppm, respectively. The experiment lasted for 90 days. Hemoglobin, packed cell volume (PCV) and total erythrocyte count (TEC) were performed as per the method described by Jain [4]. Blood biochemical study was performed by using Spectrophotometer.

**Results:** There was progressive increase in total protein, albumin, globulin levels and alkaline phosphatase activity in serum in mineral supplemented group as compare to control. In most of the cases erythrocyte count, hemoglobin (Hb) concentration and packed cell volume (PCV) were significantly (P<0.05) higher with the increase of Cr-picolinate and Zn-methionine concentration in basal diet than control. However, blood glucose and total cholesterol, triglyceride, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol in serum were significantly reduced due to mineral supplementation.

**Conclusion:** On the basis of the present experiment, it may be concluded that dietary supplementation of Zn-methionine and Cr-picolinate at the rates of 50 and 1.5 ppm, respectively had significantly improved their blood biochemical and hematological profile of desi goats.

**Keywords:** Cr-picolinate; zn-methionine; goat; blood biochemical constituents; hematological profile.

1. **INTRODUCTION**

Goat farming is one of the most important subsidiary occupations of majority of farmers in India. The livelihood of a large section of people including tribal in the country is subsistence by the goat farming. Besides, the better agro-climatic condition of the country and the ample availability of green vegetation in the forest areas favor goat rising. The trace minerals are important component of all livestock diets including goats. Chromium, in particular, is important because chromium is an active component of the glucose tolerance factor and is important in carbohydrate, protein and fat metabolism, presumably by potentiating the action of insulin [1,2]. Dietary recommendation for Cr is not listed for most livestock species including goats. Nevertheless, supplementation of Cr in livestock diets may improve growth and productive performance in non-stressed goats [3,4] and dairy heifers [5]. Zinc (Zn) is an essential element required by ruminants for a number of biochemical functions. Early work suggested that Zn deficiencies can affect growth, reproduction, immune system and gene expression in ruminants [6].

The present investigation was carried out to determine the optimum levels of inclusion of these two minerals in combination in the diet and to study their effect on blood biochemical characteristics and change in the hematological profile of goat.

2. **MATERIALS AND METHODS**

2.1 **Experimental Animal**

Twenty four non-descript healthy goat kids of 3-5 months were selected for the study. All kids were housed in a well ventilated shed with facilities for individual feeding under hygienic and uniform management conditions. All kids were sprayed with Butox (Deltamethrin) at weekly interval for three weeks before start of experiment to control the ectoparasites (ticks, mites and flies) and dewormed by Albendazole (Albendos, 2.5% w/v albendazole suspension) at the rate of 7.5 mg/kg body weight orally. The elimination of parasitic infection was confirmed by faecal examination.

2.2 **Design of Experiment**

Twenty-four kids (3-5 months old, average body weight of 5.1±0.25 Kg) of either sex were randomly allotted on body weight basis to four dietary groups. Group I (control) was fed ad libitum basal complete feed diet which constituted Indian joint vetch/sola (Aeschynomene indica) hay (60 parts) and concentrate mixture (40 parts). Group II, III and IV were fed as in the control along with a combined supplement of Zn-methionine and Cr-picolinate at the rates of 20 and 0.5 ppm, 35 and 1 ppm and 50 and 1.5 ppm respectively. Compositions of feed of different groups are presented in Table 1 and calculated chemical compositions of feeds are presented in Table 2.

2.3 **Collection of Blood**

The blood samples were collected from all kids at 0, 45 and 90 days. About 6 mL of blood was collected aseptically from each kid from jugular vein using 18 gauge needle. Out of which 2 mL blood was taken in a glass vial containing ethylene diamine tetra acetate (EDTA) for haematological studies. Another 4 mL of blood
was collected in a wide bore test tube without anticoagulant for separation of serum. The serum samples were preserved in refrigerator at 4°C for determination of biochemical parameters.

2.4 Hematological Study

Hemoglobin, packed cell volume (PCV) and total erythrocyte count (TEC) were performed as per the method described by Jain [7].

2.5 Blood Biochemical Study

Serum samples were analyzed for total serum protein, serum albumin, serum globulin, albumin: globulin ratio, cholesterol, HDL and LDL - cholesterol, alkaline phosphatase, calcium, phosphorus and triglyceride in Spectrophotometer ( Erma Inc. of Tokyo, Japan, AE-11M) and U.V. Spectrophotometer (Safas of Monaco, 4606) by using diagnostic kits (Bayer Autopk biochemistry kits - Baroda, Span Diagnostics Ltd. – Sachin, Lab-care Diagnostics Pvt. Ltd.- Sarigam) as per the methods recommended by manufacturer. Serum globulin (g/dL) was calculated by subtracting the albumin values from the values of total serum protein.

2.6 Statistical Analysis

The data of experiment in each period were analyzed for standard error and test of significance following One-way Analysis of Variance [8].

3. RESULTS AND DISCUSSION

3.1 Serum Protein Profile

The experiment demonstrated combined effects of Cr-picolinate and Zn-methionine supplementation on serum protein profile in kids (Table 3). Supplementation of Zn-methionine and Cr-picolinate in the diet resulted in significantly (P<0.05) increase in total serum protein at 45 days than control group. Among supplemented groups, group III did not differed significantly neither from group II nor from group IV. There was increase in total serum protein with increase in proportion of Cr and Zn in diet at most of the periods. It indicates that Zn and Cr both influence at the cellular level for the synthesis of nucleic acids and protein synthesis [9]. It was reflected by higher serum protein and lesser residual nitrogen, detoxified in the liver within the urea synthetic cycle. It was evident through the mechanism of chromium action for the improvement of amino acid entry into the muscular cells for the protein synthesis [10]. Our finding was also supported by Pechova [11] found significantly higher concentration of total plasma proteins in chromium supplemented in bulls. However Kitchalong et al. [12] and Ozcel et al. [13] reported that total protein concentrations was not affected due to supplementation of Cr and Zn respectively in the diet of lamb.

Supplementation of Zn methionine and Cr picolinate in the diets resulted in significant (P<0.05) rise of serum albumin concentrations in comparison to control groups in kids at 45th days however difference among all three supplemented groups were non significant statistically but the serum albumin values are higher in group II and group III as compared to group I. Critical difference test presented in table 3 indicated significantly higher serum albumin level in group IV (3.63 mg/dL) than group I (2.13 mg/dL), the difference among rest of group were non significant. The result of albumin was in accordance to the report of Moonsie-Shageer [14] that also got higher serum albumin level in Cr supplemented diet in beef. It might be due to fact that insulin being anabolic in nature help in more synthesis of amino acid in liver in the presence of Cr [15]. Neto [16] reported that Zn has direct effect on insulin synthesis and its secretion as it is a component of insulin molecule responsible for the synthesis of albumin. It was observed that supplementation of Zn and Cr in the diet significantly increased the level of globulin as compared to control group at 45days (Table 3). Although the differences among supplemented group were non-significant but progressive increase in globulin level was noticed with the increase of Zn and Cr in the diet in most cases.

3.2 Blood Glucose Level

The change in blood glucose levels in kids due to dietary supplementation of organic Zn and Cr has been given in Table 4. At 45 days, the supplementation of Zn with Cr reduced the level of glucose in group IV to 45.05 mg/dL and in group I to 54.2 mg/dL. The levels were non significant amongst group I, II and III and amongst group II, III and IV. At 90 days, the blood glucose level was significantly lower in supplemented group than control one. However, difference amongst supplemented i.e. group II, III and IV were non-significant. The low level of blood glucose may be due to relatively more synthesis of insulin in pancreas utilizing glucose for the production of energy inside the cells. This
could be achieved due to dietary supplement of Zn and Cr which might have affected the synthesis of insulin. The exact mechanism of action of Cr for the synthesis of insulin is not very clear however it enhances the binding of insulin to cell membrane receptors and optimizes the insulin activity resulting better regulation of glucose uptake by cells. Thereby improved the control of blood glucose concentration and maximize the energetic potential [17]. Zn has also direct effect on insulin synthesis and its secretion as it is a component of insulin molecule as a result more insulin is secreted which causes low blood glucose levels in Zn and Cr supplemented groups [16].

3.3 Alkaline Phosphatase Activity
There was significance difference in alkaline phosphatase activity at 45 days was recorded in group IV which was 38.18 units more than control (Table 4). There was no significant difference amongst groups I, II and III. It indicates that the optimum level of 50 ppm Zn and 1.5 ppm Cr is required to influence the level of alkaline phosphatase in serum (135.98U/l). The effect of diets had significantly influenced at 90 days also, clearly indicated that progressive increases of alkaline phosphatase activity with the increase of Cr and Zn concentration in diet. Similar trend of alkaline phosphatase activity was

| Table 1. Ingredient composition of concentrate mixture and sole (Aeschynomene indica) grass |
|---------------------------------|----------------|----------------|----------------|----------------|
| Feed ingredients | Group I | Group II | Group III | Group IV |
| Ingredient composition of concentrate mixture | | | | |
| Yellow Maize | 43 | 43 | 43 | 43 |
| Deoiled Soybean Meal | 32 | 32 | 32 | 32 |
| DORB | 22 | 22 | 22 | 22 |
| Mineral mixture | 2 | 2 | 2 | 2 |
| Salt | 1 | 1 | 1 | 1 |
| Total | 100 | 100 | 100 | 100 |
| Composition of diet (% on DM basis) | | | | |
| Concentrate mixture | 40 | 40 | 40 | 40 |
| Sole (Aeschynomene indica) grass | 60 | 60 | 60 | 60 |
| Zinc (ppm) | | | | |
| Zinc in diet | 28.78 | 28.78 | 28.78 | 28.78 |
| Zn- methionine (supplement) | 0 | 20 | 35 | 50 |
| Total Zinc | 28.78 | 48.78 | 63.78 | 78.78 |
| Chromium (ppm) | | | | |
| Chromium in diet | 0.48 | 0.48 | 0.48 | 0.48 |
| Cr-picolinate (supplement) | 0 | 0.50 | 1 | 1.50 |
| Total Chromium | 0.48 | 0.98 | 1.48 | 1.98 |

| Table 2. Chemical composition of feed ingredients and Sole (Aeschynomene indica) grass hay (% on DM basis) |
|---------------------------------|----------------|----------------|----------------|----------------|
| Particulars | Concentrate mixture | Sole grass (Aeschynomene indica) | Total (Complete mixed feed)* |
| Dry matter | 88.58 | 91 | 90 |
| Organic matter | 85.03 | 81.82 | 83.10 |
| Crude protein | 20.30 | 12.10 | 12.10 |
| Ether Extract | 2.77 | 1.19 | 1.18 |
| Crude fiber | 6.50 | 25.95 | 18.17 |
| Total Ash | 11.96 | 18.18 | 15.68 |
| Calcium | 1.37 | 2.01 | 1.99 |
| Phosphorus | 1.57 | 1.20 | 1.35 |
| Hemi cellulose | 10.47 | 16.30 | 13.97 |
| Cellulose | 6.76 | 31.36 | 21.51 |
| Lignin | 3.46 | 15.30 | 10.56 |
| Zinc (ppm) | 29.96 | 28 | 28.78 |
| Chromium (ppm) | 1.212 | 0.40 | 0.48 |
Table 3. Influence of dietary supplementation of Zn-methionine and Cr-picolinate on serum protein profiles in kids under various groups

| Period (day) | Group-I     | Group-II    | Group-III   | Group-IV    |
|--------------|-------------|-------------|-------------|-------------|
|              | Total serum protein (mg/dL) |             |             |             |
| 0            | 6.36±0.41   | 6.46±0.46   | 7.05±0.22   | 6.90±0.18   |
| 45           | 3.90±0.30a  | 5.48±0.33b  | 5.78±0.43c  | 7.10±0.19c  |
| 90           | 5.33±0.38   | 5.30±0.42   | 5.63±0.71   | 5.66±0.55   |
|              | Total serum albumin (mg/dL) |             |             |             |
| 0            | 3.35±0.23   | 3.08±0.28   | 3.86±0.23   | 3.60±0.23   |
| 45           | 2.13±0.17a  | 2.91±0.26ab | 2.75±0.29ab | 3.63±0.35p  |
| 90           | 2.68±0.30   | 2.96±0.44   | 3.25±0.49   | 2.91±0.31   |
|              | Total serum globulin (mg/dL) |             |             |             |
| 0            | 3.13±0.26   | 3.38±0.26   | 3.18±0.30   | 3.36±0.18   |
| 45           | 1.77±0.29a  | 2.57±0.20b  | 3.03±0.24ab | 3.47±0.26b  |
| 90           | 2.65±0.21   | 2.33±0.26   | 2.38±0.26   | 2.75±0.29   |
|              | Albumin: globulin ratio |             |             |             |
| 0            | 1.07±0.08   | 0.92±0.08   | 1.29±0.18   | 1.07±0.11   |
| 45           | 1.20±0.19   | 1.13±0.24   | 0.90±0.15   | 1.04±0.26   |
| 90           | 1.01±0.13   | 1.27±0.27   | 1.36±0.13   | 1.05±0.17   |

Superscripts are read row wise for comparison of mean. Different superscripts differed significantly (P< 0.05)

Table 4. Influence of dietary supplementation of Zn-methionine and Cr-picolinate on blood glucose level and alkaline phosphatase activity in kids

| Period (day) | Group-I     | Group-II    | Group-III   | Group-IV    |
|--------------|-------------|-------------|-------------|-------------|
|              | Blood glucose (mg/dL) concentrations |             |             |             |
| 0            | 54.63±1.99  | 56.16±1.98  | 54.98±3.35  | 54.36±3.10  |
| 45           | 54.2±2.24b  | 47.33±2.14ab| 47.93±3.06ab| 45.05±2.37a |
| 90           | 55.98±3.21c | 46.60±1.83c | 46.86±2.57a | 46.05±1.55a |
|              | Alkaline phosphatase activity (U/L) |             |             |             |
| 0            | 91.95±4.99  | 92.81±8.48  | 93.95±5.82  | 92.03±8.31  |
| 45           | 97.80±6.49a | 109.90±7.22a| 116.13±6.71a| 135.98±5.57b|
| 90           | 102.61±9.76a| 120.46±6.81ab| 137.90±7.16c| 161.68±11.07c|

Superscripts are read row wise for comparison of mean. Different superscripts differed significantly (P<0.05)

also found by Paul [18] in bucks of Cr supplemented group than control and it was explained that Cr augmented new bone crystal formation by enhancing the uptake of circulatory Ca into the bone matrix. It was evident by lower concentration of Ca in serum of treated groups. Zn also interfere the absorption of Ca which lowers its level in blood. In fact the low level of blood Ca stimulate parathyroid hormone to maintain the normal blood Ca by activating osteoblast cells to release alkaline phosphatase for calcification of bone. This happens especially when the animal is in the active stage of growth. In this process a little amount of alkaline phosphatase gets released in blood and elevating its level [19].

3.4 Serum Cholesterol Profile

The influence of supplemental diet had non significant influence on serum cholesterol level upto 45 days. Similarly in another study total cholesterol was not affected due to supplementation of Cr-picolinate in lamb [12]. Also, Malcolm-Callis et al. [20] found that supplementation of Zn at 20, 100 and 200 ppm in feed of beef did not affect serum cholesterol concentration. However, significantly lower serum cholesterol level was found in Cr and Zn supplemented group than control one at 90 days. However, differences in all supplemented groups were non-significant (Table 5). Almost similar to trend as observed in total serum cholesterol was also noticed in total triglyceride. Higher the level of mineral supplements lowers the level of HDL and LDL-cholesterol. There was no significant difference amongst the Zn and Cr supplemented groups. However, it was significantly different (P<0.05) from the control group.

Our finding was also supported by earlier workers [21,22,23] who reported the reduction in circulating concentrations of cholesterol and/or
non esterified fatty acids (NEFA) due to supplementation of Zn in ruminants. However in another [11] study total cholesterol was significantly reduce due to supplementation of Cr-picolinate in cattle. In current study, the decrease level of total cholesterol was due to Cr that acts through the linkage of chromodulin with the insulin receptor and increase the net synthesis of fat in adipose tissue thereby decreased its net release in serum [24]. The reduction in the triglyceride can be explained through the mechanism operating the reduction in the blood glucose level which lowers the activity of insulin and in turns releases the activity of capillary endothelial lipase causing break down of triglyceride and release of free fatty acids and glycerol [25].

### 3.5 Blood Hematological Profile

Supplementation of Zn methionine and Cr picolinate in the diets resulted in significant (P<0.05) rise of number of RBC, concentration of Hb and PCV value as comparison to control groups in kids at 45th and 90th day however difference among all three supplemented groups were non significantly statistically (Table 6). The current finding of TEC and hemoglobin concentration was in accordance to the repo rt of Onder and Kecec [26] and Schrauzer [27].

#### Table 5. Influence of dietary supplementation of Zn-methionine and Cr-picolinate on lipid profiles in kids

| Period (day) | Group-I | Group-II | Group-III | Group-IV |
|--------------|---------|----------|-----------|----------|
|              | Total serum cholesterol (mg/dL) |          |           |          |
| 0            | 58.06±4.25 | 58.33±4.47 | 60.48±5.80 | 59.43±5.46 |
| 45           | 59.20±4.17 | 51.45±3.41 | 49.81±3.56 | 50.21±4.41 |
| 90           | 61.00±4.29 | 42.93±1.97 | 34.88±2.13 | 38.01±2.56 |
|              | Total serum triglyceride (mg/dL) |          |           |          |
| 0            | 39.75±1.23 | 45.11±3.99 | 45.23±5.66 | 42.58±4.60 |
| 45           | 42.08±3.84 | 34.00±3.94 | 34.43±3.60 | 34.80±3.31 |
| 90           | 42.98±3.53 | 27.55±1.47 | 21.76±1.64 | 24.31±2.31 |
|              | Serum HDL cholesterol (mg/dL) |          |           |          |
| 0            | 4.86±0.54  | 5.45±0.29  | 5.26±0.59  | 4.96±0.47  |
| 45           | 3.18±0.35  | 2.30±0.26  | 2.45±0.34  | 2.63±0.25  |
| 90           | 5.05±0.33  | 3.23±0.18  | 2.68±0.15  | 2.76±0.28  |
|              | Serum LDL-cholesterol (mg/dL) |          |           |          |
| 0            | 45.25±3.69 | 43.86±3.45 | 46.17±4.26 | 45.95±4.09 |
| 45           | 47.60±3.12 | 42.35±2.61 | 40.48±2.52 | 40.62±3.56 |
| 90           | 47.35±3.45 | 34.19±1.52 | 27.84±1.66 | 30.38±1.95 |

*Superscripts are read row wise for comparison of mean. Different superscripts differed significantly (P< 0.05)*

#### Table 6. Influence of dietary supplementation of Zn-methionine and Cr-picolinate on hematological profiles in kids

| Period (day) | Group-I | Group-II | Group-III | Group-IV |
|--------------|---------|----------|-----------|----------|
|              | Total erythrocyte count (10^6/mm^3) |          |           |          |
| 0            | 11.34±0.39 | 11.68±0.67 | 11.01±0.52 | 11.17±0.62 |
| 45           | 8.17±0.28 | 10.50±1.03 | 11.60±0.76 | 11.84±0.44 |
| 90           | 8.60±0.52 | 10.53±1.04 | 11.82±1.48 | 12.16±0.64 |
|              | Hemoglobin concentration (g/dL) |          |           |          |
| 0            | 8.88±1.23  | 8.67±1.17  | 7.62±0.94  | 7.64±1.11  |
| 45           | 6.30±0.40 | 9.17±1.02  | 9.93±0.73  | 10.45±0.58 |
| 90           | 6.78±0.48 | 8.91±0.93 | 10.30±1.06 | 10.20±0.45 |
|              | Packed cell volume (%) |          |           |          |
| 0            | 29.78±4.01 | 27.75±1.78 | 24.64±3.15 | 24.25±3.52 |
| 45           | 19.63±0.97 | 24.98±2.45 | 30.81±1.90 | 32.91±1.85 |
| 90           | 20.90±0.86 | 30.40±0.86 | 34.36±2.87 | 31.53±1.17 |

*Superscripts are read row wise for comparison of mean. Different superscripts differed significantly (P< 0.05)*
The former reported higher level of TEC due to Zn supplementation and later higher due to Cr supplementation in the diet of beef and mice respectively. The reason may be attributed to the fact that these two minerals enhance the process of erythropoesis resulting into more formation of RBC and Hb. Although the exact mechanism of Cr is not very clear but it causes reduction in the loss of body Fe which is utilized for the synthesis of RBC and Hb [27]. Due to more RBC and high level of Hb the PCV of kids increased accordingly, in the Zn and Cr supplemented groups.

4. CONCLUSION
On the basis of the present experiment, it may be concluded that dietary supplementation of Zn-methionine and Cr-picolinate at the rates of 50 and 1.5 ppm, respectively had significantly improved their blood biochemical and hematological profile of desi goats. Significantly higher serum protein indicates more synthesis of body tissue protein in goats. Minerals increased the utilization of glucose for energy production and cholesterol for production of free fatty acids in the blood circulation.

ETHICAL APPROVAL
Animal Ethic committee approval has been collected and preserved by the author(s).

COMPETING INTERESTS
Authors have declared that no competing interests exist.

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