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Effect of Copper and Zinc Supplementation on Antioxidants and Biochemical Status of Osmanabadi Goats

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ABSTRACT

Background: Copper and zinc in animal diets significantly impact livestock productivity. Zinc is not stored in the body and must be supplemented regularly to maintain optimal immuno-physiological function in animals. Many factors restrict the bioavailability of zinc and its absorption in the intestine. Cu deficiency is common in feed ingredients. Feeding copper levels in the diet is also essential for immune system optimization since copper inhibits the development of metabolic and oxidative stress in dairy cows.

Methods: Experimental animals (n=18) were equally divided into three groups with an average body weight 29 kg. T0 was control group with basal diet, T1 group supplemented with copper sulphate (100 mg/goat/day + Basal diet) and T2 group supplemented with zinc oxide (40 mg/goat/day + Basal diet) for 90 days. Blood samples were collected at fortnight intervals and estimated using standard protocols.

Result: Total antioxidant capacity (TAC) Superoxide dismutase (SOD) and Catalase were significantly higher in zinc supplemented group followed by copper supplemented group. Thiobarbituric acid reactive substances (TBARS) were significantly lower in the zinc and copper supplemented groups compared to control group. Glucose concentration showed no significant change. Total cholesterol was significantly lower in the copper supplemented group. Total proteins, albumin and triglycerides were significantly higher in the zinc supplemented group than in other groups.

Key words: Biochemical, Catalase, Copper sulphate, Superoxide dismutase, Thiobarbituric acid reactive substance, Total antioxidants, Zinc oxide.

INTRODUCTION

Like other nutrient requirements, mineral requirements for small ruminants are not stable. Mineral requirements for goats depend upon age, sex stage and level of production. Trace mineral deficiencies are many times difficult to detect because of their symptoms that are less evident (small reductions in their average daily gains and decreased production). Zinc and copper are the second and third most essential trace minerals next to iron. Copper (Cu) and zinc (Zn) in animal diets significantly impact livestock productivity (Arangasamy *et al*., 2018).

Zinc is a nutritionally important trace element for goats since it is required for optimal feed intake and nutrient utilization, food metabolism and immunological competence (Neathery *et al*., 1973). Zinc is not stored in the body and must be supplemented regularly to maintain optimal immuno-physiological function in animals (Spears and Kegley, 2002). Zn is an anti-oxidant that reduces the production of reactive oxygen species (Bray and Bettger, 1990). Zn prevents lipids peroxidation and keeps the lysosomal membrane stable (Kimball *et al*., 1995). Zinc is a cofactor for Cu-Zn superoxide dismutase (Marklund *et al*., 1982). It plays an important role in catalase activity, regulating specificity protein 1 or other transcriptional response elements (Tate *et al*., 1995).

Copper is required for animals as a trace element. It is intimately linked to hematopoiesis, metabolism and other vital life functions (Ognik *et al*., 2016). Feeding copper levels in the diet is essential for immune system optimization since copper inhibits the development of metabolic and oxidative stress in dairy cows (Cortinhas *et al*., 2010). It is also required for defense mechanisms, iron transport, cholesterol and glucose metabolism. Although copper isn't an essential component of hemoglobin, it is found in several other plasma proteins which control iron release from cells into the plasma (Sloman *et al*., 2002).

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MATERIALS AND METHODS

Experiment was approved by Institutional Animal Ethics Committee constituted as per the article number 13 of the CPCSEA-rules, laid down by the Government of India. The present study was conducted at Osmanabadi goat farm unit, Instructional Livestock Farm Complex, College of Veterinary and Animal Sciences, Parbhani from 27 August 2021 to 25 November 2021 (90 days). Eighteen healthy adult goats were randomly selected and divided into three equal groups, T0- control group without any supplementation; T1 - group supplemented with copper sulphate (100 mg/goat/ day); T2- group supplemented with zinc oxide (40 mg/goat/ day). The diets were formulated as per ICAR (2013) recommendations. Considering their DM requirement, all the goats were stall-fed individually on concentrates and roughages.

Blood collection

Blood samples from all groups were collected in the morning hours at fortnight intervals during the study, following aseptic standards. A sterile blood vial containing a clot activator tube is used for enzyme and biochemical analysis. The serum was separated by centrifugation at 4000 rpm for 10 minutes. The serum samples were collected in micro-centrifuge tubes and stored in a deep freezer at -20°C for analysis.

Estimation of antioxidant parameters

The estimation of total antioxidants was carried out with the help of a commercially available kit. SOD, Catalase and TBARS were estimated by using standard procedures given by Marklund and Marklund (1974), Aebi (1984) and Asakawa and Matsushita (1979), respectively.

Estimation of biochemical parameters

The biochemical parameters viz. total proteins, albumin, globulin, glucose and triglyceride were analyzed by ELISA method with the help of commercially available kits. However, serum globulin was determined by mathematical calculation.

Statistical analysis

Statistical analysis was done by using one-way ANOVA, Tukey's test. IBM SPSS Statistics software version 26 was used for the data analysis.

RESULTS AND DISCUSSION

The means of antioxidant parameters such as total antioxidant, SOD, catalase and TBARS are presented in Table 1.

Total antioxidant capacity

Highly significant (P<0.01) increase was noticed in the T2 group followed by the T1 group as compared to the T0 group on TAC. The findings in the present study are in accordance with Shen *et al.* (2021) and Patel *et al.* (2021). In contrast, Ulutas *et al.* (2020) reported no significant change in antioxidant activity after zinc supplementation. This may be due to the fact that environmental and feeding factors caused no oxidative stress for the animals.

Superoxide dismutase

The SOD differed significantly (P<0.01) in the T1 and T2 groups compared to the control group. Similar findings were also reported by Patel *et al.* (2021) and Nagalakshmi *et al.* (2009) on zinc supplementation. The increase in SOD activity after zinc supplementation might be because zinc is an intrinsic constituent of superoxide dismutase, a major scavenger of free radicals present in the cytoplasm of many types of cells (Spears and Weiss, 2008). In copper supplemented groups, the present study's findings are in accordance with Zhang *et al.* (2012) and Correa *et al.* (2014).

Catalase

Significant (P<0.01) difference was noticed in T0, T1 and T2 groups. The findings were in accordance with Nagalakshmi *et al.* (2009) in catalase activity after supplementing zinc to lambs. Zinc plays an essential role in catalase activity, regulating specificity protein 1 or other transcriptional response elements (Tate *et al.*, 1995). However, copper also increases the catalase activity in Guizhou goats (Shen *et al.*, 2021).

TBARS

TBARS was significantly (P<0.01) higher in T0 followed by T1 and T2 in the present study. Similar findings were reported by Ulutas *et al.* (2020) in goats and Wei *et al.* (2019) in newborn dairy calves, zinc supplementation significantly decreased malondialdehyde (MDA) levels, which might be due to the antiperoxidative activity of zinc on lipids where the zinc stabilizes the membrane structures by antagonizing redox-active metals such as iron and copper (Shaheen and El-Fattah 1995). In the copper supplemented group, the results were in accordance with Zhang *et al.* (2012) and Shen *et al.* (2021).

Table 1: Overall means ± SE of antioxidant parameters (TAC, SOD, Catalase and TBARS) in Osmanabadi goats.

Treatment	TAC (mM)	SOD (U/ml)	Catalase (U/L)	Tbars (µM/ml)
T0	3.54^p±0.06	444.22^p±4.71	255.83^p±3.37	0.64^r±0.00
T1	4.02^q±0.06	487.08^q±5.54	301.19^q±5.10	0.58^q±0.01
T2	4.20^q±0.08	554.19^r±9.17	330.95^r±6.19	0.55^p±0.01
F Cal	27.07**	67.24**	56.62**	17.18**
P Value	0.000	0.000	0.000	0.000

^{p,q,r}Means bearing different superscripts in a column differ significantly (**P<0.01).

The means of biochemical parameters such as total protein, albumin, globulin, glucose, cholesterol and triglycerides are presented in Table 2.

Total proteins

Higher means values were noticed in the zinc supplemented group (P<0.01) as compared with T0 and T1 groups. The difference between T0 and T1 groups was non-significant. Our results are compatible with the findings of Anil *et al*. (2020) and Fagari- Nobijari *et al*. (2012). However, Sobhanirab *et al*. (2012) and Sethy *et al*. (2021) reported non-significant differences in total proteins in zinc supplemented group. Similarly, Wu *et al*. (2014) and Naseri *et al*. (2011) also reported non-significant differences in total proteins in the copper supplemented and control group, which were in accordance with our findings. The elevated serum protein levels of the zinc supplemented group in the present study indicated that zinc might have been involved in better assimilation of protein from an available dietary protein source (Grela and Pasuszak, 2004) by optimum production and activities of various proteolytic enzymes like carboxypeptidase A and carboxypeptidase B. The significantly higher total serum protein in zinc supplemented animals might have helped in maintaining well organized vital functions of proteins like maintenance of osmotic pressure of blood and tissue, the acid-base balance of blood, the activity of enzymes and peptide hormones, antibodies and clotting factors (Borah *et al*., 2014).

Albumin

Significantly (P<0.01) higher overall mean values were noticed in the T2 group, whereas the difference between T0 and T1 was non-significant (P>0.05). Our results are in accordance with Fagari-Nobijari *et al*. (2012) in Holstein bulls. Whereas, Sobhanirab *et al*. (2012) and Sethy *et al*. (2021) reported no significant differences in zinc supplementation as compared to control groups. Similarly, Naseri *et al*. (2011) and Wu *et al*. (2014) also reported no significant differences in albumin concentration after copper supplementation. The non-significant increasing trend of serum albumin from day 0 onwards till day 90 in the present experiment reflects that supplementation of zinc or zinc deficiency might not directly affect serum albumin concentration (Borah *et al*., 2014).

Globulin

No significant change was noticed between groups in the overall means. But numerically zinc supplemented group showed higher values. The improved globulin levels on zinc supplementation could be related to zinc's functional role in protein synthesis and this improved globulin level indicates a better immune response, as serum globulins play a significant role in immune response (immu nogl ob ulins or antibodies) and early line of defence (Anil *et al*., 2020).

Glucose

There was also no significant difference (P>0.05) between treatment groups in the overall means. Whereas, Sethy *et al*. (2016) reported a significant increase in glucose levels on zinc supplementation in black Bengal goats. The findings of Sethy *et al*. (2016) might be due to reduced activity of carbohydrate digesting enzymes, which are reliant on dietary zinc levels, which might cause the decreased blood glucose levels in zinc supplemented groups. However, Hesari *et al*. (2012) found no significant increase in glucose concentrations after copper supplementation, which was in accordance with our findings. Copper is required for defence mechanisms, iron transport, cholesterol and glucose metabolism and brain development (Felltman, 1991).

Total Cholesterol

Highly significant (P<0.01) decrease was noticed in the T1 group compared with T2 and T0 groups. Our findings are in agreement with the results of Samanta *et al*. (2011) and Zhang *et al*. (2012). However, our findings of zinc supplementation when compared with the control group are similar to the results reported by Ulutas *et al*. (2020) and Sobhanirab *et al*. (2012) who found no significant changes in cholesterol levels. Nobijari *et al*. (2012) reported a significant decrease in cholesterol after zinc supplementation in Holstein bulls. They further stated that this might be indirectly due to the decreased availability of blood glucose in zinc supplemented groups because glucose also acts as a source for cholesterol production in the biological system. While Sung and Dale, (1981) claimed that Zn has a positive correlation with blood cholesterol levels.

Table 2: Overall means ± SE of biochemical parameters (total proteins, albumin, globulin, glucose, total cholesterol and triglycerides) in osmanabadi goats.

Treatment	Total proteins (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Glucose (mg/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)
T0	8.40^{p}±0.07	3.50^{p}±0.03	4.88±0.8	54.90±0.56	64.35^{q}±1.24	110.54^{p}±1.07
T1	8.42^{p}±0.08	3.47^{p}±0.04	4.96±0.10	53.59±0.63	59.81^{p}±0.87	113.11^{p}±1.06
T2	8.81^{q}±0.09	3.77^{q}±0.04	5.04±0.09	54.74±0.79	64.47^{q}±0.88	116.35^{q}±0.99
F cal	7.31**	14.70**	0.68NS	1.13NS	6.79**	7.70**
P value	0.001	0.000	0.50	0.32	0.002	0.001

^{p,q,r}Means bearing different superscripts in a column differ significantly (**P<0.01).

^{NS} - Non-significant.

Triglycerides

Significantly (P<0.01) higher mean values were observed in the T2 group, followed by T1 and lower values in the T0 group. The difference between T0 and T1 groups remained non-significant. The findings of present investigation are similar to the results published by Sobhanirab *et al*. (2012) in cows, in the zinc supplemented group. This might be because zinc plays a direct role in the regulation of lipid metabolism and has structural and functional characteristics of lipid enzymes (Ulutas *et al*., 2020). While Zhang *et al*. (2012) and Guclu *et al*. (2008) reported no significant difference in triglyceride levels between copper supplemented and control group.

CONCLUSION

The supplementation of copper sulphate and zinc oxide showed a positive effect on antioxidants parameters (TAC, SOD, Catalase and TBARS), which can act as an excellent supplement to goats during stress conditions. Supplemen tation of zinc and/or copper can boost immunity because globulin was higher in the treatment groups. Total proteins, albumin, total cholesterol and triglycerides were significantly higher in zinc supplemented groups. Globulin and glucose were non-significantly higher in zinc supplemented group as compared to other groups. All the biochemical parameters are within normal limits.

Conflict of interest: None.

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