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Effect of copper and zinc supplementation on hematological parameters of osmanabadi goats

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Abstract
The present study was conducted on (n=18) Osmanabadi Goats for 90 days. Experimental animals were equally divided into three groups (T0, T1, and T2) with an average body weight of 29 kg. T0 was the control group and offered only a basal diet; the T1 group was supplemented with copper sulphate (100 mg/goat/day + Basal diet), and the T2 group was supplemented with zinc oxide (40 mg/goat/day + Basal diet). Blood samples were collected at fortnight intervals. Results indicated that MCHC values were significantly higher; Hb, RBC, and MCH values were non-significantly higher, whereas WBC and PCV values were non-significantly lower in the copper supplemented group. RBC and PCV values were non-significantly higher, whereas Hb and MCH values were significantly lower in the zinc supplemented group as compared with the control group. DLC revealed significantly higher lymphocytes, whereas neutrophils and monocytes were lacking in the zinc-supplemented group.

Keywords: Copper sulphate, haematology, osmanabadi goats, zinc oxide

1. Introduction
Mineral requirements for small ruminants are not stable. They depend upon age, sex stage and level of production. Trace mineral deficiencies are often difficult to detect because their symptoms 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. By regulating the function of T-cells and B-cells, zinc plays a vital role in the immune system [1]. Zn's immune-stimulatory impact is mainly derived from its participation in lymphocyte and natural killer cell activity and cytokine and lymphokine production [2]. It is also required to develop and exercise neutrophils and natural killer cells, which mediate non-specific immunity. Zinc is needed for better outgrowth and function of T- lymphocyte, B-lymphocyte development, antibody production – particularly immunoglobulin G and macrophage function [3]. Carbonic anhydrase (found in RBC) contains 0.3 percent zinc required for CO2 removal. Zinc also plays a role as a cofactor in blood coagulation, fibrinolysis, hemostasis, and thrombosis [4].
Cu is necessary for developing antibodies and white blood cells, along with antioxidant enzyme production [5]. Although copper isn't an essential component of hemoglobin, it is found in several other plasma proteins, including ceruloplasmin, which controls iron release from cells into the plasma [6]. Copper is required for animals as a trace element. It is intimately linked to hematopoiesis, metabolism, development, reproduction, and other vital life functions, and it has the ability to effectively regulate the stability of the internal environment [7].

2. Materials and Methods
Institutional Animal Ethics Committee approved an experiment constituted as per article number 13 of the CPCSEA-rules, laid down by the Government of India. The present study was conducted at the 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 (100mg/goat/day); T2 – group supplemented with zinc oxide (40mg/goat/day). The diets were formulated as per ICAR (2013) recommendations. All the goats were stall-fed individually on concentrates and roughages, considering their DM requirement. They were given an adaptation period of 10 days before the trial and followed by a treatment period of 90 days.

2.1 Blood collection
Blood samples from all groups were collected in the morning hours at fortnight intervals during the study, following aseptic standards. Sterile blood vial EDTA for hematological analysis. The samples were immediately carried in the thermal box containing ice packs to the laboratory for research.

2.2 Hematological parameters
All the hematological parameters were analyzed (hemoglobin (Hb), total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count (DLC), and packed cell volume (PCV)) by a hematology analyzer. Whereas mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) were calculated by the standard formula.

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

3. Results and Discussion
The means of hematological parameters are provided in Table 1. Significantly (P<0.01) higher Hb concentration was recorded in the copper supplemented group and the control group compared to the zinc supplemented group. The findings of the zinc supplemented group are in accordance with Sobhanirad *et al*. [8] and Ulutas *et al*. [9] in Holstein dairy cows compared to control groups. Here in the present study, the hematological parameters were in the normal range, which might be due to the level of inclusion of zinc that might not have interfered with the Cu and Fe to affect the hemogram. Contrast findings were reported by Naseri *et al*. [10] in dairy calves, Solaiman *et al*. [11], and Rodriguez *et al*. [12] in goat kids. They reported that the Hb concentration was higher in the control group than the copper supplemented group. This might be due to the effect of hemolytic anemia caused by copper toxicity, which would reduce the Hb concentration in lambs [13].

RBC showed no significant variations between groups in the present study. But numerically increase in RBC values was recorded in both copper and zinc supplemented groups as compared to the control group. Our results are in accordance with Heidarpour *et al*. [14] in neonatal dairy calves, Solaiman *et al*. [11] in goat kids, Naseri *et al*. [10] in dairy calves, Shen *et al*. [15] in Guizhou black goats and Rodriguez *et al*. [12] in beef cows of copper supplemented group. Whereas Anil *et al*. [16] in crossbred calves, Ramulu *et al*. [17] in Murrah buffalo calves, Elamin *et al*., [18] in goat kids and Azizzadeh *et al*. [19] in neonatal dairy calves reported no significant change in RBC after zinc supplementation.

WBC also showed no significant difference between the treatment and control groups. But decreased WBC values were recorded in copper supplemented groups compared to other groups. Results in the present study of zinc supplemented group is in accordance with Ramulu *et al*. [17] in Murrah buffalo calves, Sobhanirad *et al*. [8] in Holstein dairy cows, Anil *et al*. [16] in crossbred calves, Elamin *et al*. [18] in goat kids and Ulutas *et al*. [9] in goats. However, Naseri *et al*. [10] in dairy calves, Solaiman *et al*. [20] in goats, Heidarpour *et al*. [14] in dairy calves, and Rodrigues *et al*. [12] in beef cows also reported non-significant differences in WBC count in copper supplemented group. According to Solaiman *et al*. [11], leukocytosis is usually a consequence of an increase in the total number of circulating neutrophils, although other cells may also be increased in some circumstances. An increase in leukocyte count in Cu supplemented group of goats may indicate a stress reaction; however, these values fall within the normal range [21].

No significant differences were noticed in PCV % between the control and treatment groups. But numerically, the zinc group showed higher values and the copper group showed lower values than the control group. A similar type of observation was also reported by Naseri *et al*. [10] in dairy calves and Rodriguez *et al*. [12] in beef cows. Both the authors reported non-significant differences in PCV of the copper supplemented group. Whereas Shen *et al*. [15] noted a significant increase in PCV in copper supplemented goats. In zinc supplemented groups, our findings were in accordance with Ulutas *et al*. [9], Ramulu *et al*. [17] and Elamin *et al*. [18]; they reported no significant differences in PCV. However, Sobharinad *et al*. [8] observed a considerable increase in PCV% on zinc methionine supplementation in Holstein dairy cows.

No significant alterations was noticed on MCV in between treatment groups. But overall means of T1 and T2 groups were lower than the T0 group. The findings of the study are in agreement with Naseri *et al*. [10], Heidarpour *et al*. [14] and Shen *et al*. [15] in the copper supplemented group. Similarly, Sobhanirad *et al*. [8], Ramulu *et al*. [17], Azizzadeh *et al*. [19] and Anil *et al*. [16] reported no significant differences in MCV during zinc supplementation.

MCH showed significant (P<0.05) differences in the zinc supplemented group compared with control groups. The values of the present study are within the standard limit [21] but little higher than that of Shen *et al*. [15]. However, Sobhanirad *et al*. [8] and Azizzadeh *et al*. [19] found no significant differences in MCH values on zinc supplementation. Similarly, in copper supplemented groups, Heidarpour *et al*. [14] and Shen *et al*. [15] reported no significant changes in MCH values which were in line with our findings. An MCH value refers to the average quantity of haemoglobin present in a single red blood cell. Haemoglobin is the protein in red blood cells that transports oxygen to all the body's tissues. High MCH values are a common sign of macrocytic anemia, and low values indicate less Hb in red cells.

Significantly (P<0.01), higher values of MCHC were observed in the T1 group than other groups, whereas T0 and T2 groups remained non-significant but MCHC was non-significantly more elevated in the T2 group. However, Naseri *et al*. [10] and Shen *et al*. [15] reported non-significant differences in MCHC on copper supplementation.

Significantly lower values were noticed on neutrophils in the zinc supplemented group (P<0.01) compared with the control and copper supplemented group. Non-significantly higher values are observed in T0 as compared to T1 group. The mean values obtained in the present study are similar to Solaiman *et al*. [20]. Whereas, Solaiman *et al*. [11] reported higher values than the present study. However, Naseri *et al*.[10] said no significant change in neutrophils of the copper supplemented group and control group, which is in accordance with the present study. But Heidarpour *et al*. [14] and Solaiman *et al*. [11] observed a significant difference in neutrophil %. Bharadwaj *et al*. [22] reported a decrease in neutrophils on zinc administration, which was in accordance with our findings. Whereas, Ulutas *et al*. [9] reported a significant increase in neutrophils on zinc supplementation in goats compared to the

control group, which contradicts the present study. Non-infectious disease conditions resulting in leucocytosis or neutrophilia are usually a result of a stress reaction [23]. Neutrophils are important effector cells in the innate arm of the immune system [24]. They constantly patrol the organism for signs of microbial infections, and when found, these cells quickly respond to trap and kill the invading pathogens.

A highly significant increase in lymphocytes was noticed in the zinc supplemented group ($P<0.01$) followed by the copper supplemented group compared to the control group. However, Naseri *et al.* [10] and Heidarpour *et al.* [14] reported no significant change in lymphocytes on copper supplementation. Similarly, Sobhanirad *et al.* [8] and Ramulu *et al.* [17] found no significant difference in lymphocytes on zinc supplementation. Zinc is a nutritionally important trace element for goats; it is required for optimal feed intake and nutrient utilization, growth and skeletal development, hair and skin integrity, reproductive, food metabolism, and immunological competence [25, 26]. By its role in the function of T-cells and B-cells, zinc, copper, and iron play a vital role in the immune system [1]. Zn's immune-stimulatory impact is mainly derived from its participation in lymphocyte and natural killer cell activity and cytokine and lymphokine production [2]. In the present study, it is observed that the lymphocytes are higher in copper and zinc supplemented groups, but the values are within the normal range in all the groups [21]. Significantly higher values of lymphocytes in zinc and copper supplemented groups indicate that the immune system is boosted in T1 and T2 compared to the T0 group.

Monocytes were significantly ($P<0.01$) higher in T0 and lower in the T2 group than in T1. Similar mean values were reported by Solaiman *et al.* [11] in goats which support the present investigation findings. Similarly, other researchers [10, 14] reported no significant monocyte variation on copper supplementation. However, Sobhanirad *et al.* [8] and Ramulu *et al.* [17] observed a non-significant difference in monocytes in the zinc supplemented group. Monocytes are white blood cells; like other white blood cells, monocytes are essential in the immune system's having the ability to destroy the invaders. This proves that the immune system is excellent in experimental goats.

Non-significantly higher mean values of eosinophils were observed in T0 followed by T1, as compared to the T2 group. Our findings are in line with Naseri *et al.* [10] and Heidarpour *et al.* [14]. They also reported no significant change in eosinophils on copper supplementation. Similar findings of non-significant difference in zinc supplemented group were reported by various researchers [8, 17]. Eosinophils are principal effective cells in the immune system. They have a beneficial role in host defence against parasitic infections and are active participants in many immune responses. However, eosinophils can also be damaging as part of the inflammatory process of allergic disease. It is confirmed by the values of the present study that the experimental goats are free from any type of parasitic infections and allergic conditions because the values obtained in the current investigation are in the normal range [21].

Similarly, basophils also showed non-significantly higher values in the T1 group followed by T0 compared to the T2 group in the present study. Our findings agree with Naseri *et al.* [10] and Heidarpour *et al.* [14]. They reported no significant change in basophils on copper supplementation. Similarly, findings were also reported by other researchers in the zinc supplemented group [8, 17]. Basophils play an essential role in "immune surveillance". This means that they have the ability to detect and destroy some early cancer cells. Another important function of basophils is that they release the histamine in their granules during an allergic reaction. In the present study, it is proved that the immune system of the experimental goat is strong because the results obtained in the antioxidant parameters accelerate the antioxidant activity, which supports the present study that the supplementation of copper and zinc is beneficial to maintain the health status of the animals.

Table 1: Overall means ± SE of haematological parameters (Hb, RBC, WBC, PCV, MCV, MCH, MCHC, DLC) in Osmanabadi goats

Treatment	Hb (gm%)	RBC ($\times10^6$/ mm^3)	WBC ($\times10^3$/ mm^3)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/L)	Neutro (%)	Lympho (%)	Mono (%)	Eosino (%)	Baso (%)
T0	$11.19^{p}\pm0.10$	8.91 ± 0.24	8.28 ± 0.08	33.6 ± 0.67	38.9 ± 1.24	$12.88^{q}\pm0.33$	$33.71^{p}\pm0.64$	$33.19^{q}\pm0.54$	$56.76^{p}\pm0.54$	$5.69^{q}\pm0.19$	4.28 ± 0.19	0.04 ± 0.03
T1	$11.48^{p}\pm0.12$	9.45 ± 0.16	8.05 ± 0.09	32.31 ± 0.67	35.57 ± 0.84	$12.28^{pq}\pm0.24$	$36.11^{q}\pm0.81$	$31.57^{q}\pm0.54$	$59.07^{q}\pm0.54$	$5.42^{pq}\pm0.22$	3.88 ± 0.16	0.09 ± 0.04
T2	$10.80^{q}\pm0.10$	9.31 ± 0.21	8.26 ± 0.10	34.23 ± 0.62	37.92 ± 1.13	$11.85^{p}\pm0.27$	$32.12^{p}\pm0.59$	$29.61^{p}\pm0.55$	$61.71^{r}\pm0.71$	$4.88^{p}\pm0.19$	3.71 ± 0.23	0.02 ± 0.02
F Cal	8.86^{**}	1.16^{NS}	1.30^{NS}	2.24^{NS}	2.54^{NS}	3.29^{*}	8.50^{**}	10.61^{**}	16.61^{**}	3.97^{*}	2.23^{NS}	1.05^{NS}
P Value	0.000	0.314	0.27	0.11	0.08	0.04	0.000	0.000	0.000	0.021	0.11	0.35

Means bearing different superscripts in a column differ significantly

*$P<0.05$ – Significant

**$P<0.01$ – Highly significant

^{NS} Non-significant

4. Conclusion

The haematological values are in the normal physiological limits; copper and/or zinc supplementation did not affect the CBC and health status of the goats. Supplementation of zinc and copper can boost immunity because lymphocytes were higher in the treatment groups.

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