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Effect of nickel supplementation on the antioxidant and immune status of growing lambs

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ABSTRACT

The present investigation aimed to explore the impact of nickel (Ni) supplementation on the antioxidant and immune status of growing Muzaffarnagari lambs. For the study, 18 healthy Muzaffarnagari lambs (30.45±3.67 kg) body weight and age (10.33±0.67 months) were randomly divided into 3 groups of 6 each. The composition of basal diet was similar across all three groups, except for Ni supplementation at dose level of 0.0 (control), 1.5 ($Ni_{1.5}$), and 3.0 ($Ni_{3.0}$) mg/kg DM, administered to the respective groups over a 90-day study period. Blood samples were collected by jugular puncture on days 0, 15, 30, 45, 60, 75, and 90 of the study in the morning, before feeding and watering. The superoxide dismutase (SOD), total antioxidant activity (TAA), and thiobarbituric acid reactive substance (TBARS) did not vary statistically among the groups, control, $Ni_{1.5,}$ and $Ni_{3.0}$, whereas, catalase activity declined significantly ($P<0.05$) in groups that received 1.5 or 3.0 mg Ni/kg DM as compared to the control group. Nickel supplementation did not influence the concentration of haemoglobin (Hb), total leucocyte count (TLC), and neutrophils. Lymphocyte concentration was statistically ($P<0.05$) higher in lambs who received Ni at a dose of 3.0 mg/kg DM as compared to other animals. Plasma levels of total immunoglobulin (TIG) were higher in treatment groups of lambs fed either 1.5 or 3.0 mg Ni/kg DM than in the control group. The findings of the present study indicated that nickel supplementation at both doses (1.5 and 3.0 mg/kg DM) improved immune function; however, it did not significantly affect the antioxidant status of growing lambs.

Keywords: Antioxidant status, Gmmune status, Growing lambs, Nickel

Minerals are integral components of numerous vitamins, enzymes, hormones, and other essential nutrients required for the growth and development of animals. They function as cofactors in various physiological processes, including metabolism, immunity, and reproduction. Even moderate mineral deficiencies can adversely affect animal health and performance. Recently, Ni has been recognized as a trace element with potential beneficial effects on animal health. It is considered a relatively newly identified essential trace mineral, and its deficiency has been associated with increased susceptibility to metabolic stress and positive physiological responses to supplementation (Nielsen, 2000). Ni is believed to be involved in the activation of enzymes and hormones, modulation of oxidative stress and immune function, and regulation of protein, lipid, and carbohydrate metabolism (La Bella *et al.* 1973). Additionally, serves as a prosthetic component of SOD (Ni-SOD), which protects cells from oxidative damage by catalyzing the dismutation of superoxide radicals into hydrogen peroxide and oxygen (Jason, 2014). Ni supplementation has been found to improve catalase activity in buffalo calves (Thamizhan *et al.* 2025) and enhanced the activities of SOD and catalase in rats (Sidhu *et al.* 2004), however, it reduced TAA concentrations in Hariana calves (Singh *et al.* 2019). In contrast, Das *et al.* (2001) reported that a high dietary level of Ni (75 mg Ni/kg DM) significantly increased lipid peroxidation while decreasing the activities of antioxidant enzymes. Despite these roles, the precise mechanisms underlying immunomodulatory effects of Ni remain unclear, particularly given reports of immune impairment associated with excessive nickel exposure as a heavy metal. An optimum dose of nickel supplementation improved immune function, possibly by enhancing ruminal activity through increased urease enzyme function (Singh *et al.* 2019), modulating immune cell responses, and alleviating stress via reduced cortisol concentrations (Thamizhan *et al.* 2025), whereas higher levels may exert immunosuppressive effects (Das *et al.* 2001).

Therefore, the present study was designed to investigate the impact of dietary Ni supplementation on antioxidant and immune responses in growing lambs.

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MATERIALS AND METHODS

Ethics approval: The procedure and protocols of the study were approved by the Institutional Animal Ethics Committee and CPCSEA, Government of India (V-11011 (13)/19/2020/CPCSEA-DADF).

Animal, diet, and feeding: The experiment was conducted at the Instructional Livestock Farm Complex–2, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India. A total of 18 healthy Muzaffarnagari lambs with similar body weight (30.45 ± 3.67 kg) and age (10.33 ± 0.67 months) were selected and randomly allocated into three groups, with six lambs in each group. The composition of diet was same in all three groups except for Ni which was supplemented additionally at dose rates of 0.0 (N_0), 1.5 ($N_{1.5}$), and 3.0 mg/kg DM ($N_{3.0}$) in the respective groups in the form of Ni sulfate heptahydrate ($NiSO_4.7H_2O$, Purity 98%, SRL, Pvt. Ltd., Mumbai, India) for 90 d of the study period. The ratio of concentrate, straw, and green fodder in the diet was 50:30:20, respectively. Diet was formulated (Table 1)as per the recommendation given by ICAR (2012) and provided twice daily *ad libitum* at 08.00 h and 17.00 h, and had free access to fresh drinking water. The weighed dose of Ni was mixed with a small amount of concentrate and given to each lamb separately.

Table 1. Ingredients and chemical composition of the diet offered over the experimental period

Ingredients	Content (g/kg DM)
Ingredient composition of basal diet	
Berseem	150
Oat fodder	200
Wheat straw	200
Ground maize	200
Wheat bran	40
Pulses chuni	50
Mustard cake	150
Mineral and vitamin premix[a]	10
Nickel sulfate heptahydrate	Variable*
Chemical Composition	
Dry matter	580
Crude Protein	145
Ether Extract	290
Total ash	90
Neutral detergent fiber	470
Acid detergent fiber	290

[a] Composition of premix per kg: Vitamin A 7 lac IU, vitamin D_3 3 lac IU, alpha-tocopherol 300 IU, Vitamin B_{12} 6 mg, nicotinamide 1.0 g, Cu 1.2 g, Cobalt 40 g, Fe 8 g, Zn 15 g, Mg 6 g, Iodine 0.33 mg, Se 0.001 g, Mn 20 g, Ca 300 g, P 140 g, S 0.72 g, Na 20 g, and K 0.1 g

*1.5 and 3.0 ppm Ni were acquired by adding 1.5 and 3.0 mg Ni/kg DM as nickel sulfate heptahydrate (NiSO4. 7H2O, purity 98%, SRL Pvt. Ltd., Mumbai) in the basal diet.

Sample collection: Blood was collected from jugular vein in a test tube, containing EDTA as an anticoagulant, before offering feed and water on 0, 15, 30, 45, 60, 75, and 90 d of the experimental period. Fresh blood sample was taken to assess Hb, TLC, neutrophils, and lymphocytes counts, after which the remainder was centrifuged at 3000 rpm for 30 minutes in a refrigerated centrifuge to obtain plasma. Plasma samples were kept in 2.0 ml round-bottomed Eppendorf tubes and stored at -20 °C until total antioxidant activity (TAA), Thiobarbituric acid reactive substances (TBARS), and total immunoglobin level (TIG) were further analyzed. Hemolysate was prepared from haematocrit and subsequently analyzed for SOD and catalase activities.

Sample analysis: Hemolysate was quantified for activities of SOD (Marklund and Marklund, 1974) and catalase (Aebi, 1984). The TAA levels were quantified by ferric reducing antioxidant power method, as explained by Benzie and Strain (1999). The protocol of Feldman *et al.* (2000) was used for TLC in the blood. Neutrophil and lymphocyte counts were performed by using a cross-sectional technique (Schalm and Jain, 1986). The extent of lipid peroxidation (in terms of TBARS) was measured by the TBA test method (Asakawa and Matsushita, 1979). The plasma concentration of TIG was estimated using Zn turbidity method (McEwan and Fisher, 1970).

Statistical analysis: The data obtained for antioxidant and immune status were analyzed using a MIXED model with repeated measures in IBM SPSS Statistics (IBM Corp., Armonk, NY, USA). The model was used as follows-

$$Y_{ij} = \mu + C_i + G_j + e_{ij}$$

Where Y_{ij} is the dependent variable, μ is the overall mean of the population, C_i is the random effect of calves, G_{jj} is the fixed effect of treatment (j = 0, 1.5, and 3.0 mg/kg DM), and e_{ij} is the residual element.

Tukey's Honestly Significant Difference, a post hoc test, was used to compare the treatment means on different days of the study. Orthogonal polynomial contrasts were also analyzed and there were two (no. of groups - 1) for the present experiment, first-order contrasts compute linear relationships, and second-order contrasts compute quadratic relationships.

RESULTS AND DISCUSSION

Antioxidant status: The observations related to antioxidant status at different intervals are presented in Table 2. The mean activities of SOD and TAA, as well as TBARS levels, were unaffected by Ni supplementation at doses of 1.5 or 3.0 mg/kg DM across the experimental fortnights. In contrast, the mean catalase activity, as well as its values were significantly lower ($P < 0.05$) in the group fed 3.0 mg/kg DM Ni during the 2nd, 4th, 5th, and 6th fortnights of the study period as compared to the other groups.

SOD, catalase, and TAA are key biomarkers used to assess the antioxidant defense system. SOD converts superoxide

Table 2. Effect of nickel supplementation on antioxidant status in plasma of growing Muzaffernagari lambs (n = 6) over a 90-day trial period

Variable	Fortnight	Treatment			SEM	P value		
		N_0	$N_{1.5}$	$N_{3.0}$		Contrast	Linear	Quadratic
Superoxide dismutase (U/mg Hb)	0	2.14	2.16	2.18	0.56	0.986	0.869	0.978
	1	2.16	2.19	2.13	0.66	0.982	0.926	0.872
	2	2.20	2.22	2.19	0.40	0.985	0.953	0.873
	3	2.18	2.20	2.26	0.47	0.943	0.744	0.929
	4	2.21	2.23	2.06	0.51	0.788	0.577	0.693
	5	2.20	2.15	2.28	0.37	0.764	0.66	0.560
	6	2.22	2.07	2.04	0.46	0.700	0.438	0.756
	Mean	2.15	2.17	2.16	0.52	0.963	0.784	0.993
Catalase (µmol H_2O_2 consumed/min/g Hb)	0	0.31	0.29	0.28	0.03	0.326	0.480	0.924
	1	0.34	0.32	0.28	0.05	0.056	0.022	0.509
	2	0.35^b	0.34^b	0.27^a	0.03	<0.001	<0.001	0.011
	3	0.36	0.35	0.30	0.07	0.270	0.128	0.607
	4	0.37^b	0.36^b	0.31^a	0.04	0.003	0.002	0.156
	5	0.36^b	0.35^b	0.30^a	0.03	0.001	0.001	0.046
	6	0.37^a	0.40^{ab}	0.29^b	0.05	0.002	0.007	0.006
	Mean	0.35^b	0.34^b	0.29^a	0.04	<0.001	<0.001	0.001
Total antioxidant activity ($\times10^3$µmol/L)	0	1.25	1.27	1.30	0.05	0.242	0.098	0.873
	1	1.27	1.30	1.33	0.08	0.411	0.191	0.889
	2	1.29	1.33	1.35	0.13	0.645	0.369	0.828
	3	1.32	1.31	1.34	0.11	0.875	0.743	0.695
	4	1.35	1.34	1.37	0.12	0.888	0.758	0.712
	5	1.34	1.36	1.31	0.13	0.806	0.671	0.624
	6	1.33	1.35	1.33	0.15	0.929	0.940	0.711
	Mean	1.31	1.32	1.33	0.11	0.538	0.275	0.828
Thiobarbituric acid reactive substance (nmol/ml)	0	1.38	1.35	1.33	0.06	0.311	0.138	0.782
	1	1.39	1.39	1.39	0.14	0.999	0.984	0.972
	2	1.42	1.40	1.35	0.08	0.297	0.133	0.729
	3	1.47	1.38	1.36	0.10	0.086	0.035	0.509
	4	1.44	1.44	1.45	0.15	0.977	0.833	0.968
	5	1.41	1.42	1.40	0.14	0.958	0.919	0.788
	6	1.40	1.41	1.39	0.16	0.981	0.903	0.880
	Mean	1.41	1.40	1.38	0.12	0.383	0.167	1.000

N_0, control group without Ni supplementation; $N_{1.5}$, nickel supplemented group with dose 1.5 mg/kg DM; $N_{3.0}$, nickel supplemented group with dose 3.0 mg/kg DM; Contrast, the contrast effect of nickel supplementation; Linear, linear effect of nickel; Quadratic, the quadratic effect of nickel; SEM, Standard error mean.

Mean bearing different superscripts (a, b) in a row showed a significant difference at P<0.05

radicals into hydrogen peroxide, while catalase further breaks it down into water and oxygen, preventing oxidative damage. TAA reflects the overall antioxidant capacity of the body while, TBARS indicates lipid peroxidation and serves as a marker of oxidative damage. Different doses of Ni supplementation did not affect the SOD and TAA activities, or TBARS levels in growing lambs; however, a reduction in catalase activity was observed. In agreement with the present findings, Singh *et al.* (2019) reported no significant effect of Ni supplementation at 1.5 or 3.0 mg/kg DM on SOD, TAA, and TBARS in growing Hariana heifers. In earlier reports, Ni supplementation had no effect on TAA and glutathione peroxidase activities in crossbred dairy calves receiving 10 mg/kg DM Ni (Shambhvi *et al.* 2023) and in buffalo calves supplemented with 5 or 10 mg/kg DM Ni (Thamizhan *et al.* 2025). Farid *et al.* (2012) had also reported that Ni supplementation through drinking water (180 mg/L) reduced catalase activities in rats. However, Das *et al.* (2001) reported increased hepatic lipid peroxidation along with decreased activities of SOD, catalase and glutathione peroxidase in mice supplemented with 75 mg/kg DM Ni, which contrasted with the present

Table 3. Effect of nickel supplementation on immunological status of growing Muzaffernagari lambs (n = 6) over a 90-day trial period

Variable	Fortnight	Treatment			SEM	P value		
		N_0	$N_{1.5}$	$N_{3.0}$		Contrast	Linear	Quadratic
Hemoglobin (g/100 ml)	0	11.86	11.88	11.90	0.18	0.984	0.861	0.995
	1	11.94	12.16	12.18	0.19	0.663	0.419	0.696
	2	12.79	11.98	11.99	0.33	0.147	0.091	0.313
	3	13.43	12.64	12.83	0.32	0.169	0.166	0.190
	4	12.98	13.51	12.99	0.40	0.511	0.980	0.253
	5	13.39	13.65	13.70	0.26	0.817	0.560	0.818
	6	12.90	12.51	12.65	0.32	0.749	0.627	0.565
	Mean	12.60	12.62	12.61	0.25	0.703	0.450	0.718
Total leukocyte count ($\times 10^3/\mu l$)	0	7.79	7.82	7.85	0.61	0.981	0.849	0.984
	1	8.51	8.13	8.35	0.65	0.490	0.611	0.285
	2	7.96	7.98	7.69	0.67	0.609	0.411	0.583
	3	8.61	8.09	8.13	0.69	0.233	0.158	0.334
	4	7.76	7.58	7.66	0.69	0.867	0.710	0.661
	5	8.44	8.46	8.18	0.66	0.635	0.433	0.599
	6	7.95	7.82	7.96	0.52	0.835	0.996	0.554
	Mean	7.96	7.98	7.97	0.65	0.295	0.164	0.478
Neutrophil (%)	0	25.57	25.71	25.86	1.34	0.913	0.673	1.000
	1	26.14	26.29	26.43	1.92	0.952	0.757	1.000
	2	27.00	27.14	27.29	1.80	0.946	0.743	1.000
	3	26.57	26.86	26.86	1.79	0.928	0.740	0.848
	4	27.29	27.29	27.43	1.69	0.980	0.864	0.921
	5	28.00	26.86	28.14	1.36	0.133	0.832	0.049
	6	27.71	28.43	27.57	1.38	0.439	0.841	0.211
	Mean	26.90	26.94	27.08	1.61	0.833	0.566	0.854
Lymphocyte (%)	0	61.00	61.29	61.43	1.11	0.709	0.422	0.876
	1	61.71	61.86	62.00	1.46	0.919	0.685	1.000
	2	62.14	62.29	61.86	1.21	0.756	0.628	0.576
	3	61.57	63.14	62.43	1.56	0.132	0.260	0.090
	4	62.14	62.86	63.57	1.50	0.158	0.058	1.00
	5	63.00	62.57	63.71	1.29	0191	0.254	0.152
	6	61.71^{a}	63.71^{b}	62.86^{ab}	1.51	0.043	0.136	0.037
	Mean	61.93^{a}	62.53^{ab}	62.55^{b}	1.37	0.029	0.019	0.201
Total immunoglobulin (mg/ml)	0	31.11	31.14	31.16	0.72	0.989	0.884	0.986
	1	31.87	31.95	31.99	1.50	0.985	0.868	0.973
	2	32.33	32.97	33.02	1.90	0.702	0.456	0.710
	3	33.05	34.98	33.73	1.54	0.225	0.186	0.260
	4	32.58^{a}	33.97^{ab}	35.08^{b}	1.29	0.010	0.003	0.823
	5	33.05^{a}	35.99^{b}	34.00^{ab}	1.83	0.011	0.297	0.004
	6	34.04^{a}	36.98^{b}	36.20^{b}	1.06	<0.001	0.001	0.001
	Mean	32.58^{a}	34.00^{b}	33.81^{b}	1.48	0.005	0.013	0.035

N_0, control group without Ni supplementation; $N_{1.5}$, nickel supplemented group with dose 1.5 mg/kg DM; $N_{3.0}$, nickel supplemented group with dose 3.0 mg/kg DM; Contrast, the contrast effect of nickel supplementation; Linear, the linear effect of nickel; Quadratic, the quadratic effect of nickel; SEM, Standard error mean.

Mean bearing different superscripts (a, b) in a row showed a significant difference at $P<0.05$.

results. Shambhvi *et al.* (2023) and Thamizhan *et al.* (2025) reported higher catalase activity in Ni-supplemented groups, which may be attributed to differences in dosage levels and animal species.

Immune status: The mean TLC, neutrophil count, and Hb concentrations in the blood and their values across the different fortnights did not vary statistically among the groups (Table 3), which indicated that Ni supplementation

altered the blood concentrations of Hb, TLC, and neutrophils significantly. The mean lymphocyte count was significantly higher ($P < 0.05$) in lambs receiving 3.0 mg/kg DM Ni compared to the control group at 6th fortnight; however, their value did not differ significantly from that of lambs receiving 1.5 mg/kg DM Ni. Similar findings were also observed for total immunoglobulin contents.

In the present study, different levels of Ni supplementation did not affect TLC, neutrophil count, or Hb concentration; however, lymphocyte count and plasma TIG concentrations increased. Shambhvi *et al.* (2023) had also reported no change in TLC with dietary Ni supplementation at 5.0, 7.5, or 10 ppm in crossbred dairy calves. Similarly, Thamizhan *et al.* (2025) reported no significant effect of Ni supplementation with 5 or 10 mg/kg DM on TLC, which is in agreement with the present study; however, they observed an increase in Hb concentration, which contrasted with the current findings. The current study indicated that Ni supplementation at 1.5 or 3.0 mg/kg DM enhanced the immune status of growing lambs, with a greater response observed in 1.5 mg/kg DM Ni-fed lambs, possibly due to increased lymphocytes in the blood, as reported in the present study. Another possible reason for the improved immunity in lambs receiving Ni supplements might be that Ni might function as a vital cofactor for the enzyme rumen urease, boosting urease activity (Spear *et al.* 1977), enhancing protein utilization, and promoting nitrogen recycling in ruminants (Singh *et al.* 2019), lowering the stress via reducing cortisol (Thamizhan *et al.* 2025) which ultimately supports the immune system's metabolic needs. However, Singh *et al.* (2019) and Shambhvi *et al.* (2023) observed no significant effect of Ni supplementation on TIG and IgG levels in dairy calves, suggesting no influence on immune response.

The results of the present investigation indicated that Ni supplementation did not affect SOD, TAA, TBARS, Hb, TLC, or neutrophil levels; however, it increased lymphocyte count and total immunoglobulin concentrations, suggesting an improvement in the immune status of growing sheep supplemented with 1.5 or 3.0 mg/kg DM Ni.

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REFERENCE

Aebi H. 1984. Catalase *in vitro*. *Methods in Enzymology* **105**: 121–126.

Asakawa T and Matsushita S. 1979. Coloring condition of thiobarbituric acid test for detecting lipid hydroperoxide. *Lipids* **15**: 137–140

Benzie I F F and Strain J J. 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology* **299**: 15–27

Das K K, Das S N and Das Gupta S. 2001. The influence of ascorbic acid on nickel- induced hepatic lipid peroxidation in rats. *Journal of Basic and Clinical Physiology and Pharmacology* **12**: 187–194.

Farid H E, Abozid M M and Mahmoud, K E. 2012. short-term effects of vanadium and nickel intoxication on rats liver antioxidant defence system. *International Journal of Academic Research* **4**(5): 23–28.

Feldman B F, Zink J G, Jain N C and Schalm O W. 2000. *Schalm's veterinary hematology*, 5th edn. Lippincott Williams and Wilkins, Philadelphia

ICAR. 2012. *Nutrient Requirement of Sheep, goat and Rabbit*-2, Indian Council of Agriculture Research, New Delhi, India

Jason S. 2014. Insight into the structure and mechanism of nickel-containing superoxide dismutase derived from peptide-based mimics. *Accounts of Chemical Research* **47**: 2332–2341

La Bella F S, Dular R, Lemons P, Vivian S and Queen M. 1973. Prolactin secretion is specifically inhibited by nickel. *Nature* **245**: 330–332

Marklund S and Marklund G. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry* **47**: 469–474.

McEwan A D and Fisher E W. 1970. A turbidity test for the estimation of immunoglobulins levels in neonatal calf plasma. *Clinica Chemica Acta* **17**:155–165.

Nielsen F H. 2000. Importance of making dietary recommendations for elements designated as nutritionally beneficial, pharmacologically beneficial, or conditionally essential. *Journal of Trace Element and Experimental Medicine* **13**: 113–129.

Schalm O W and Jain N C. 1986. *Schalm's veterinary haematology*, 4th edn. Lea and Febiger, Philadelphia

Shambhvi, Datt C, Thamizhan P, Chauhan P, Dudi K and Mani V. 2023. Effects of nickel supplementation on nutrient utilization, mineral balance, hematology and antioxidant status of crossbred dairy calves. *Journal of Trace Elements in Medicine and Biology* **79**: 127250.

Sidhu P, Garg M L and Dhawan D K. 2004. Protective role of zinc in nickel induced hepatotoxicity in rats. *Chemico-Biological Interactions* **150**: 199–209.

Singh A, Kumar M, Kumar V, Roy D, Kushwaha R, Vaswani S and Kumar A. 2019. Effects of nickel supplementation on antioxidant status immune characteristics and energy and lipid metabolism in growing cattle. *Biological Trace Element Research* **190**: 65–75.

Spears J W, Smith C J and Hatfield E E. 1977. Rumen bacterial urease requirement for nickel. *Journal of Dairy Science* **60**:1073–1076.

Thamizhan P, Datt, C, Shambhvi, Chauhan P, Thakuria A and Malik R. 2025. Influence of supplementary nickel on minerals balance, hematobiochemical parameters, antioxidant activity, plasma minerals and hormones status in Murrah buffalo calves. *Agriculture Research* **14**: 559–568.