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Effect of Zinc supplementation on haematology, oxidative stress and plasma biochemical parameters in cadmium exposed goats

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ABSTRACT

The present study was designed to assess the effect of zinc supplementation on haematology, oxidative stress and plasma biochemical parameters in Cd induced lactating goats. Eighteen crossbred lactating goats were divided into three groups (Control, T_1 and T_2). In T_1 and T_2 groups, goats were given 10 ppm $Cdcl_2$, while in T_2, Zinc @100ppm was also supplemented for period of 120 days. At the end of study, blood glucose, TLC, neutrophil % and AST, ALT were increased significantly in T_1 group and except glucose, Zn supplementation in T_2 group showed its protective effect on TLC, neutrophil %, lymphocyte %, AST and ALT. There was significant decrease in lymphocyte %, Hb and plasma total antioxidant activity in T_1 group. At 30 days, the activity of SOD was increased in T_1 and T_2 but thereafter activities were significantly decreased at 120 days in T_1 but in T_2 it was similar to control. The activity of catalase was increased on 30 and 60 days and thereafter decreased from 90 days onwards but the rate of decline was comparatively less in T_2. Total immunoglobulin and lymphocyte stimulation index were significantly decreased in T_1 group whereas in Zn supplemented group decline trend was less. Henceforth, it was concluded that 100 ppm Zn is helpful in reducing burden of Cd induced biochemical and oxidative stress in goats.

Keywords: Catalase, Glucose, SOD, Total immunoglobulin, Total antioxidant activity

Due to technological advancement in industry and agriculture, many toxic heavy metals, pesticides and herbicides are being added to the environment, which are of concern because of their persistence in ecosystem (Hayat *et al*. 2019). Since, heavy metals have low threshold for toxicity, continuous exposure even at the low doses may affect health and performance of living beings. Cadmium (Cd) is one of the most toxic elements which have no nutritional role in biological system. Its exposure is associated with wide range of organ toxicity (Khan *et al*. 2017). Body lacks an effective homeostatic control mechanism against Cd and it is having a long biological half-life of 10-30 years (Morsy *et al.* 2020). All these factors contribute to its harmful nature towards animals and human beings. The primary route of exposure to Cd in domestic animals is via ingestion of contaminated herbages grown over contaminated soil. Previously Cd toxicity in domestic animals was rather rare, but exposure to cadmium is gradually increasing, especially in grazing animals by ingestion of Cd taken up by plants (Hooser 2018). Fitzgerald *et al*. (1985) reported that Cd was the only metal to accumulate consistently in increased amounts in the tissues of the cattle when they were allowed to graze pastures treated with anaerobically digested sewage sludge for up to 8 years. It has also been reported that cattle grazing on sewage sludge-treated pastures consumed three times more Cd than cattle on control pastures (Reddy and Dorn 1985). Cai *et al*. (2009) showed that in China, around lead-zinc smelter area (soil with 10 ppm), cattle (feed with 6.6 ppm Cd) had higher level of Cd in kidneys (38 mg/kg) and liver (2.5 mg/kg). This is because of accumulation of Cd in tissues upoun chronic exposure as body has no mechanism for the excretion of Cd. Liver and kidney remain the primary target organ upon long-term exposure to Cd (Patra *et al.* 2007). Cd is known to disturb the oxidative status of the body. The mechanism of the Cd-induced oxidative stress is not fully known. Although Cd does not generate free radicals, it elevates lipid peroxidation in tissues as well as changes in the activities of antioxidant enzymes, including copper-zinc-manganese superoxide dismutase (SOD) and catalase (Rahimzadeh *et al*. 2017). Most cadmium in the body is bound by metallothionein which also binds essential metals such as copper and zinc (Lisic *et al.* 1996). Zn is found to be antagonist with Cd (NRC 2007). The antagonism of Zn and Cd cannot be explained by Cd reducing Zn in tissues. However, feeding 350 ppm to calves decreased Zn absorption, but the effect was independent of the lowered feed intake and nitrogen balance which resulted from Cd feeding. Cd displaces Zn in many vital enzymatic reactions, causing disruption or

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cessation of enzymatic activities, in turn, supplementation of trace mineral, i.e. Zn, Cu could enhance immunity by maintaining functional and structural integrity of important immune cells (EI Demerdash *et al*. 2004). In sheep, more than 5 ppm Cd in diet causes increased Zn concentration in liver and kidney (Underwood and Suttle 2001). Considering the above stated facts, in the present study 10 ppm dose of Cd is employed to study its cumulative pattern.

Although the mechanism of Cd toxicity has been extensively studied on laboratory animals, there are limited number of studies on ruminants. Therefore, the present study was designed to investigate the effect of Zn on oxidative stress and biochemical parameters in Cd exposed lactating goats.

MATERIALS AND METHODS

Eighteen crossbred lactating goats (2^{nd} to 3^{rd} lactation) were selected from the Cattle Yard of National Dairy Research Institute, Karnal, India, and distributed randomly into three groups of six each. The distribution of the animals was done on the basis of average milk yield (1.66±0.02 kg/day) and body weight (41.17±0.79 kg). The total duration of the experiment was 120 days. The dietary treatment were control, basal diet without Cd supplementation; T_1, Cd @10 mg/kg dietary DM as Cd chloride ($CdCl_2.H_2O$, Merck Limited, Mumbai, India, minimum assay ≥98% purity); T_2, Cd 1@10 mg/kg dietary DM with Zn 100 mg/kg DM as $ZnSO_4$ ($ZnSO_4.7H_2O$, S d fine-Chem Limited, India, minimum assay 99.5%).

The gelatin capsules containing weighed quantity of Cd and Cd plus Zn were prepared and fed on daily to animals in group T_1 and T_2, respectively. The animals were given experimental diet after an adaptation period of one week. The experimental diets were made as per feeding standard of NRC (1981). Chemical composition of the diet is presented in Table 1. After taking approval from Institutional Animal Ethics Committee (NDRI, India), experiment was conducted.

Blood samples collected in two sets of heparinized vacuitainer tube (Becon Dickinson India Pvt. Ltd., New Delhi, India); one for haematological and another for biochemical study. Blood was collected before initiation of trial and thereafter at 30 days intervals. Plasma was harvested subsequently by centrifuging the whole blood samples at 840 g for 15 min in a portable centrifuge machine.

Table 1. Chemical composition of feeds

Parameter	Concentrate mixture	Berseem fodder
Crude protein (%)	19.94	15.80
Total ash (%)	8.88	10.80
Ether extract (%)	3.20	2.52
Crude fibre (%)	8.26	25.50
Organic matter (%)	91.50	89.20
Nitrogen free extract (%)	59.72	45.38
Zinc (ppm)	39.60	15.10
Cadmium (ppm)	0.30	0.22

The heparinized plasma samples were stored at −20°C in storage vials and analyzed subsequently. The haemoglobin (Hb) content of blood was estimated by Drabkin Cyanmethemoglobin Method (1994). Total leukocyte count (TLC), blood lymphocyte % and neutrophil % were done using hemocytometer. Glucose was analyzed using Glucose test kits (Span Diagnostics Ltd., Surat, India). Plasma AST and ALT was estimated using commercial kit. Total immunoglobulin was estimated by precipitation method of McEwan and Fisher (1970). Total antioxidant activity was measured by ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1999). Superoxide dismutase (SOD) was measured following the method of Marklund and Marklund (1974). Catalase (CAT) activity in RBC hemolysate was estimated following the method of Aebi (1984). The proliferative response of lymphocyte was estimated using the colorimetric 3-(4-5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) according to the procedure given by Mosmann (1983). Stimulation index is a ratio of optical density of stimulated and unstimulated cells. The data were analysed using two-way repeated measures ANOVA with two factors, i.e. different treatment groups and periods (using SigmPlot 11 ® software package (Systat software Inc., USA). The results were represented as mean±SE. The model used for the data analysis was as follows:

$$Y_{ijk} = \mu + G_i + D_j + G*D_{ij} + e_{ijk}$$

Where, Y_{ijk}, observation associated with each parameters; μ, overall mean; G_i, effect of i^{th} group (i=1, 2 and 3); D_j, effect of j^{th} days (j=1, 2, 3, 4 and 5); $G*D_{ij}$, interaction effect of j^{th} days within i^{th} group and e_{ijk}, random errors.

RESULTS AND DISCUSSION

There was no significant effect (P>0.05) of dietary treatments on Hb up to 90 days of experiment whereas on 120^{th} day, there was significant (P=0.015) decline in Hb content in T_1 group compared to control (Table 2). There was no significant interaction (P=0.165) between treatment and period. Up to 60 days, there was no significant effect (P>0.05) of dietary Cd supplementation on blood TLC count (million/mm). From 90 days onwards, TLC was increased significantly (P=0.028) in T_1 groups than control, whereas, in T_2 groups increase was less as compared to T_1 and the values were comparable to control which indicated mitigating potential of Zn. Cd supplementation significantly (P=0.016) increased the blood neutrophil (%) while Zn supplementation was able to reduce this increased trend as the final values were statistically at par with control values. There was decline trend of lymphocyte % in T_1 (P=0.002) and T_2 groups and Zn supplementation was able to mitigate the decline trend (P=0.012) significantly at the end of experiment.

Cd administration had an adverse effect on erythropoiesis. Therefore, decrease Hb in the present study may be due to increased rate of RBC destruction and hyperactivity of bone marrow that resulted in production of red blood cells with impaired integrity (Miladenovic

Table 2. Haematological changes in cadmium intoxicated goats

Parameter	Days	Control	T_1	T_2	SEM	P-value
Haemoglobin (g%)	0	9.87	9.76	9.70	0.05	NS
	30	10.85	10.67	10.65	0.06	NS
	60	10.88	10.71	10.68	0.06	NS
	90	10.86	9.98	10.63	0.26	NS
	120	10.42^b	8.97^a	9.30^a	0.44	0.015
TLC ($X10^3$ Cmm^{-1})	0	10.42	9.97	10.30	0.13	NS
	30	10.55	10.45	10.32	0.07	NS
	60	10.87	11.46	10.62	0.25	NS
	90	10.17^a	11.98^b	10.42^a	0.57	0.004
	120	10.13^a	12.27^b	10.68^a	0.64	<0.001
Neutrophil (%)	0	27.33	28.50	27.50	0.36	NS
	30	32.33	35.00	33.50	0.77	NS
	60	33.17	36.33	34.33	0.92	NS
	90	32.33^a	38.17^b	34.83^{ab}	1.69	0.006
	120	34.00^a	42.67^b	36.00^a	2.62	<0.001
Lymphocyte (%)	0	71.50	69.67	70.67	0.53	NS
	30	67.00	63.67	65.33	0.96	NS
	60	65.17	61.50	63.67	1.06	NS
	90	66.67^b	60.50^a	63.33^{ab}	1.78	0.015
	120	63.50^b	55.67^a	62.00^b	2.40	0.002

Superscript bearing a,b values in column show significant (P<0.05) effect due to treatment.

et al. 2014). The results are at par with Oraby *et al.* (2015) and Bayoumi *et al.* (2013) who observed decrease in Hb concentration in Cd intoxicated sheep. Supplementation of Zn @100 ppm to Cd exposed goats prevented the decrease in the Hb concentration in the blood up to 60 days effectively and this may be due to reduction in copper and Zn absorption by the Cd supplementation. The results of TLC are in accordance with Bose (1999) who reported increase in blood TLC count in goats exposed to Cd @150 mg/day through drinking water but in contrary, Oraby *et al.* (2021) found decrease in TLC. The literature in ruminants on this aspect is very meager but on comparing the results with monogastric animal similar trend is reported. EI Demerdash *et al.* (2004) found increased blood TLC count in Cd exposed male rats. This increase in TLC might be due to increased number of neutrophils. The observations on neutrophils are similar to Bose (1999) who observed significant increase of neutrophil count in chronic Cd exposed lactating goats. Present findings are supported by Guilhermino *et al.* (1998) and Lafuente *et al.* (2004) who reported decrease in lymphocyte in peripheral blood of adult male rat on being exposed to 10 ppm Cd.

From 90 days onwards, blood glucose concentration (mg/100ml) was increased significantly (P<0.001) in T_1 and T_2 groups compared to control and Zn supplementation did not reduce the values significantly (Table 3). Throughout

Table 3. Blood biochemical profiles of arsenic treated and control goats

Parameter	Days	Control	T_1	T_2	SEM	P-value
Glucose (mg/dl)	0	51.01	50.82	50.83	0.06	NS
	30	50.31	50.51	50.54	0.07	NS
	60	51.04	54.22	51.25	1.31	NS
	90	50.49^a	58.33^b	55.25^b	1.56	<0.001
	120	50.85^a	67.15^b	64.74^b	3.00	<0.001
AST (U/L)	0	93.21	94.75	92.60	0.64	NS
	30	93.55	95.71	93.86	0.67	NS
	60	94.11	105.74	99.02	3.37	NS
	90	102.89	117.01	103.53	4.60	NS
	120	105.91^a	130.91^b	118.64^{ab}	7.22	0.011
ALT (U/L)	0	8.42	8.44	9.16	0.24	NS
	30	8.85	8.39	9.33	0.27	NS
	60	8.48	10.78	9.89	0.67	NS
	90	8.44^a	11.11^b	10.43^{ab}	0.80	0.016
	120	9.95^{ab}	12.20^b	8.44^a	0.92	0.014

Superscript bearing a,b values in column show significant (P<0.05) effect due to treatment.

Table 4. Activity of SOD (Units/g/Hb/min), catalase (μmol of H_2O_2 consumed/min/mg Hb), total antioxidant activity (μmol/L) and total immunoglobulin (mg/ml) in blood of control and experimental goats

Parameter	Days	Control	T_1	T_2	SEM	P-value
SOD	0	1854.09	1859.987	1853.07	2.16	NS
	30	1867.78	1864.49	1860.69	2.05	NS
	60	1865.34	1841.967	1839.76	7.66	NS
	90	1867.65	1743.88	1769.59	41.70	NS
	120	1853.86^b	1651.17^a	1852.16^b	46.98	<0.001
Catalase	0	136.47	137.70	136.40	0.42	NS
	30	135.32	139.36	139.69	1.41	NS
	60	135.42	147.35	145.15	3.59	NS
	90	135.04	125.76	124.57	3.31	NS
	120	134.92	119.57	122.83	4.67	NS
Total antioxidant activity	0	1040.71	1046.90	1066.31	7.71	NS
	30	1051.85	1052.37	1072.64	6.84	NS
	60	1107.42	1076.80	1094.31	8.87	NS
	90	1192.77^b	1036.86^a	1053.00^a	49.50	0.035
	120	1191.28^b	997.08^a	1016.87^a	61.70	0.004
Total immunoglobulin	0	23.65	23.47	23.09	0.16	NS
	30	23.66	22.40	23.31	0.38	NS
	60	22.90	22.14	23.49	0.39	NS
	90	23.26	21.23	23.60	0.74	NS
	120	23.23^b	18.89^a	21.90^b	1.28	0.009

Superscript bearing a,b values in column show significant (P<0.05) effect due to treatment.

the experiment there was increased trend of plasma AST and ALT activities (U/l) and at 120 days, compared to control, higher (P<0.05) activities were found in T_1 group. Similarly, T_2 groups showed significant (P<0.05) protective effect against both AST and ALT, by low increase in their activities.

In current experiment, increase in blood glucose might be due to increased secretion of glucocorticoids. These findings are in agreements with Orabi *et al.* (2015) who attributed the increase in glucose level due to decrease in glucose utilization and disruption in insulin and glucagons hormone. The increase in plasma AST and ALT activities indicated an active transamination of amino acids. Therefore, increase in the activities of AST and ALT in plasma is mainly due to the destructive effects of Cd on liver and kidney tissues and consequently liberating their intra cellular enzymes into the blood stream (Stoev *et al.* 2003), which gives an indication of the hepatotoxic effect of $CdCl_2$. This is similar to findings of Orabi *et al.* (2021) and Patel *et al.* (2009). While, in contrast, Erdogan *et al.* (2005) reported that Cd (25 mg/l) administration did not results in significant changes in AST and ALT in broilers. Zn is one of the nutrients that can reduce the toxicity of orally consumed Cd and shows its effect by competing with Cd for the same transport system as well as for the binding sites in the metallothionine (McDowell 1992).

On the day 120th, significant (P<0.05) inhibition (11%) of SOD activity was observed in T_1, however, in T_2 group it was similar to control. Therefore, Zn supplementation was able to mitigate the adverse effect of Cd (Table 4). After an initial rise of catalase activity up to 60 days, there was significant decrease in the activity in both T_1 (P=0.007) and T_2 (P=0.045) groups at 120 days, however, between these two groups no difference was observed. Plasma total antioxidant activity (μmol/l) significantly (P=0.006) decreased in T_1 group and Zn supplementation did not significantly (P=0.941) recover that activity at 120 days. Total immunoglobulin concentration (mg/ml) were significantly (P<0.001) decreased in T_1 group at 120 days and supplementation of Zn was able to increase this value. Average lymphocyte stimulation index at the end of 120 days was significantly (P=0.001) lower in T_1 group as compared to control and T_2 due to Cd supplementation (Fig. 1 and Table 5) and between treatment and period there was no significant interaction (P=0.069).

Zn played a protective role in counteracting the adverse effects of Cd on SOD as seen in T_1 group. These findings are in agreement with the report of Orabi *et al.* (2021) and Bayoumi *et al.* (2013) who observed significant decrease in SOD activity in sheep exposed to Cd. Amara *et al.* (2008) reported that Zn administration restored the SOD activity in testis of the Cd exposed rat. On the contrary, Han

Table 5. Effect of Zn supplementation on lymphocyte proliferation index in Cd exposed lactating goats

Day	Control	T_1	T_2	SEM	P-Value
0	0.80	0.82	0.79	0.01	NS
40	0.74	0.77	0.74	0.01	NS
80	0.95^{ab}	0.78^a	1.15^b	0.11	0.003
120	0.92^{ab}	0.74^a	1.14^b	0.12	0.001

Superscript bearing a,b values in column show significant (P<0.05) effect due to treatment.

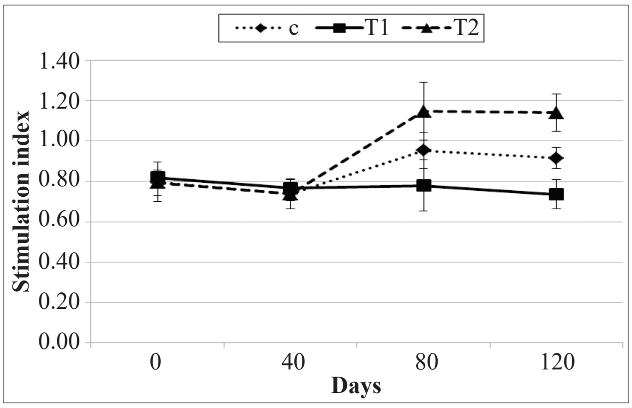

Fig. 1. Effect of Zn supplementation on lymphocyte proliferation in Cd exposed lactating goats.

et al. (2005) observed that SOD and catalase activity was not changed in pigs fed with 0.5 mg/kg Cd. The increase in catalase activity on day 60 may be due to increase in catalase synthesis to mitigate oxidative stress as self defence mechanism (Pi *et al.* 2002). But subsequently, reduced activity of catalase enzymes in Cd supplemented group was observed which might be due to down regulated synthesis or over-utilization of antioxidant enzyme resulting failure of adaptive mechanism. Also, Zn supplementation did not restore catalase activity. These findings are comparable with Amara *et al.* (2008) in rat. The decrease in total antioxidant activity in Cd supplemented group might be due to increased production of reactive oxygen metabolites resulting oxidative stress. Chauhan and Agrawal (1999) reported significant reduction in total immunoglobulin in Cd exposed calves. Kundu (1993) reported that when calves supplemented with Cu and Zn, there was increase in humoral immune response (IgG) thus confirming immuno-modulatory effect of Zn. Cd interferes with the functioning of necessary elements like Zn in enzyme system leading to its deleterious effect on immunity of man and animals (EI-Demerdash *et al.* 2004). Lymphocyte stimulation is widely used to measure immune competence by stimulation of lymphocytes with phyto-mitogens. These results are in agreement with Kundu (1993) who reported increased cellular immune response in milk fed calves supplemented with Cu and Zn. Zn plays an important role in cell-mediated immunity through its involvement in cell replication and proliferation (Bartoskewitz *et al.* 2007).

The results suggest that long term exposure of Cd is detrimental to the animals as it induces severe oxidative stress, decreases cellular immunity and causes alteration in different haemato-biochemical parameters. Moreover, supplementation of Zn may be helpful to combat Cd induced ill effects in dairy goats.

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REFERENCES

Aebi H.1984. Catalase *in vitro*, pp. 121–26. *Methods in Enzymology.* (Ed) Packer L, New York.

Amara S, Abdelmelek H, Garrel C, Guiraud P, Douki T, Ravanatalain J L, Favier A, Sakly M and Rhouma K B. 2008. Preventive effect of zinc against cd-induced oxidative stress in the rat testis. *Journal of Reproduction and Development* **54**: 129–34.

Bartoskewitz M L, Hewitt D G, Laurenz J C, Pitts J S and Bryant F C. 2007. Effect of dietary copper and zinc concentrations on white-tailed deer antler growth, body size, and immune system function. *Small Ruminant Research* **73**: 87–94.

Bayoumi Y H. 2013. 'Assessment of the impact of environmental pollution with heavy metals on sheep reared at Bahr El Bakar region.' PhD Thesis. Faculty of Veterinary Sciences, Zagazig University.

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

Bose. 1999. 'Toxico and immunopathological effects of induced chronic Cd intoxication in goats.' M.V.Sc. Thesis, West Bengal University of Animal and Fishery Sciences, Calcutta, India.

Cai Q, Long M L, Zhu M, Zhou Q Z, Zhang L and Liu J. 2009. Food chain transfer of cadmium and lead to cattle in a lead-zinc smelter in Guizhou, China. *Environmental Pollution* **57**(1): 3078–82.

Chauhan R S and Agrawal D K. 1999. Immunopathology of Cd in bovine calves. *Journal of Immunology and Immunopathology* **1**: 31–34.

Drabkin D L. 1944. *Photometry and spectrometry: Medical physics,* Vol. 1. Year Book Medical Publishers Inc., Chicago.

El Demerdash F M, Yousef M I, Kedwany F S and Baghdadi H H. 2004. Cd-induced changes in lipid peroxidation, blood haematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and β-carotene. *Food and Chemical Toxicology* **40**: 1563–71.

Erdogan Z, Erdogan S, Celik S and Unlu A. 2005. Effects of ascorbic acid on Cd-induced oxidative stress and performance of broilers. *Biological Trace Element Research* **104**: 19–32.

Fitzgerald P R, Peterson J and Lue Hing C. 1985. Heavy metals in tissues of cattle exposed to sludge-treated pastures for eight years. *American Journal of Veterinary Research* **46**: 703–07.

Guilhermino L, Soares A M V M, Carvalho A P and Lopes M C. 1998. Effects of Cd and Parathion exposure on haematology and blood biochemistry of adult male rats. *Bulletin of Environmental Contamination and Toxicology* **60**: 52–59.

Han X, Xu Z, Wang V and Huang G. 2005. Effect of cadmium on lipid peroxidation and activities of antioxidant enzymes in growing pigs. *Biological Trace Element Research* **110**: 251–63.

Hayat M T, Nauman M, Nazir N, Ali S and Bangash N. 2019. In: Cadmium toxicity and tolerance in plants, pp. 163–83. *Environmental Hazards of Cadmium: Past, Present, and Future*. (Ed) Mirza H. Elsevier.

Hooser S B. 2018. Cadmium, pp. 417–421. *Veterinary Toxicology*. Academic Press.

Khan M A, Khan S, Khan A and Alam M. 2017. Soil contamination with cadmium, consequences and remediation using organic amendments. *Science of The Total Environment* **601-602**: 1591–1605.

Kundu M S. 1993. 'Dietary zinc and copper levels influencing immune status of calves.' Ph D. Thesis., National Dairy

Research Institute (Deemed University), Karnal, India.

Lafuente A, Gonz´alez Carracedo A and Esquifino A I. 2004. Differential effects of Cd on blood lymphocyte subsets. *Biometals* **17**: 451–56.

Lisic R C, Sarras M P, Hidalgo J and Andrews G K. 1996. Metallothioncin is a component of exocrine pancreas secretion: implications for zinc homeostasis. *American Journal of Physiology-Cell Physiology* **271**: C2204–C1110.

Marklund S and Marklund S. 1974. Involvement of superoxide dismutase anion radical in autoxidation of pyrogallol and a convenient assay for Superoxide dismutase. *European Journal of Biochemistry* **42**: 469.

Mladenovic J, Ognjanovic B, Dordevic N, Matic M, Knezˇevic V, Stajn A and Saicˇic Z. 2014. Protective effects of oestradiol against cadmium-induced changes in blood parameters and oxidative damage in rats. *Archives of Industrial Hygiene and Toxicology* **65**: 37–46.

McDowell L R. 1992. *Minerals in Animal and Human Nutrition*. pp. 359–61. Academic Press, New York.

Mc Ewan A D and Fisher E W. 1970. A turbidity test for the estimation of immunoglobulins levels in neonatal calf serum. *Clinica Chimica Acta* **17**: 155.

Morsy M M, El Ghannam A A and Saleh S Y. 2020. Assessment of serum mineral concentrations of barki sheep and its impact on kidney functions in El-Hammam city. *Advances in Animal and Veterinary Sciences* **8**: 68–75.

Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* **65**: 55–63.

NRC. 1981. Nutrient requirements of goats: Angora, dairy and meat goats in temperate and tropical countries. National Academy Press, Washington, D.C.

NRC. 2007. Nutrient requirements of small ruminants: Sheep, goats, cervids and new world camelids. National Academy Press, Washington, DC.

Oraby M I, Rakha G H and Elsayed A A. 2015. 'Some studies on effect of drainage water on sheep health.' M.Sc. Thesis. Faculty of Veterinary Medicine, Cairo University, Egypt.

Orabi M I, Baraka T A and Rakha G H. 2021. Impact of cadmium intoxication on health status, rumen and blood constituents in Egyptian Ossimi Sheep. *International Journal of Veterinary Sciences* **10**: 102–06.

Patel A K, Kewalramani N, Mani V and Kaur H. 2009. Effect of vitamin E supplementation in growing kids fed on arsenic containing diet. *Indian Journal of Animal Nutrition* **26**: 9–16.

Patra R C, Swarup D, Naresh R, Kumar P and Nandi D. 2007. Tail hair as an indicator of environmental exposure of cows to lead and cadmium in different industrial areas. *Ecotoxicology and Environmental Safety* **66**: 127–31.

Pi J, Yamauchi H and Kumagai Y. 2002. Evidence for induction of oxidative stress caused by chronic exposure of Chinese residents to arsenic contained in drinking water. *Environmental Health Perspective* **110**: 331–36.

Rahimzadeh M R, Rahimzadeh M R, Kazemi S and Moghadamnia A A. 2017. Cadmium toxicity and treatment: An update. *Caspian Journal of Internal Medicine* **8**: 135–45.

Reddy C S and Dorn C R. 1985. Municipal sewage sludge application on Ohio farms: Estimation of cadmium intake. *Environmental Research* **38**: 377–88.

Stoev S D, Grozeva N, Simeonov R, Borisov I, Hubenov H, Nikolov Y, Tsaneva M and Lazarova, S. 2003. Experimental cadmium poisoning in sheep. *Experimental and Toxicologic Pathology* **55**: 309–14.

Underwood E J and Suttle N F. 2001. Essentially toxic elements (aluminium, arsenic, cadmium, fluorine, lead, mercury), pp.543–586. *The Mineral Nutrition of Livestock*. (Eds) E J Underwood and N F Suttle . CABI Publishing, New York, USA.