



Ameliorative effect of zinc supplementation to lead exposed goat kids on lymphocyte proliferation and superoxide dismutase (SOD) expression

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ABSTRACT

In vivo studies were conducted to observe the adverse effects of lead and protective effect of zinc on lymphocyte proliferation and SOD expression in 18 crossbred (Alpine × Beetal) male goat kids. They were divided into 3 groups i.e. group 1 (control), group 2 (control + 50 ppm Pb) and group 3 (control + 50 ppm Pb + 50 ppm Zn). All the kids were fed as per standard dietary requirements for 90 days. Blood samples were collected on 0, 30, 60 and 90 days of Pb and Zn supplementation for lymphocyte separation. A fixed no. of cells (2×10^6) was grown in culture for 72 h for studying the lymphocyte proliferation and SOD expression. There was no effect of dietary treatments on feed intake of the animals. Overall average lymphocyte proliferation response at the end of 90 days duration was significantly lower in Pb supplemented group 2 as compared to groups 1 and 3. The adverse effect of lead on lymphocyte proliferation was recovered to some extent by Zn supplementation, but, it was still significantly less than the control, indicating that Zn addition in the diet of Pb exposed kids could not fully recover the animals from the adverse effect. SOD expression remained almost similar in group 1 during 90 days period, but substantial decrease in relative abundance of SOD at 60 days was observed in Pb supplemented group 2 which further decreased at 90 days of Pb exposure. The decrease in SOD expression was recovered to some extent in goat kids of group 3 supplemented with Zn.

Key words: Goat kids, Lead, Lymphocyte proliferation, Real time PCR, Superoxide dismutase (SOD) expression, Zinc

Presence of lead in the diet of animals can lead to oxidative stress and depress the immunity status resulting thereby in poor productive and reproductive performance (Liu and Kuch 2005). Lead is also known to alter expression of certain genes involved in defense mechanism against oxidative stress specially activities of SOD (Gurer and Ercal 2000). Zn and Pb compete for similar binding sites on a metallothionin-like transport protein, and that the presence of Zn reduces absorption of Pb from the gastrointestinal tract (Flora and Tandon 1990). Diets low in Zn can further increase the oxidative stress and adversely affect immunity status of animals. Supplementation of Zn in the diet of heavy-metals exposed animals can help to reduce the adverse effect of lead, and improve the blood lymphocyte population, phagocytosis and killing ability by macrophages. Therefore, this study was conducted to examine the effect of zinc

supplementation to lead exposed goat kids on lymphocyte proliferation and superoxide dismutase (SOD) expression.

MATERIALS AND METHODS

Crossbred (Alpine × Beetal) male goat kids (18) were selected from the NDRI herd and randomly divided into group 1 (control), groups 2 and 3, which were administered 50 ppm Pb, and group 3 was also supplemented with 50 ppm Zn in the diet to counter the adverse effects of Pb. The treatments were continued for 90 days. All the kids were fed as per NRC (1981) requirements. The nutrient requirements of kids were met by feeding concentrate mixture and lucerne (*Medicago sativa*) fodder. Concentrate mixture consisted of groundnut cake 21 parts, maize 33 parts, wheat bran 20 parts, rice bran 11 parts, de-oiled mustard cake 12 parts, mineral mixture 2 parts and common salt 1 part. Chemical composition of feeds offered to goat kids during the experimental period of 90 days is presented in Table 1. The body weight at the start of the experiment averaged 8.92 ± 1.4 , 9.14 ± 0.6 , 8.93 ± 0.6 kg in the 3 respective groups. The feeds offered and residue left were recorded daily to find out the total DM intake of the animals.

Blood samples were collected at 0, 30, 60 and 90 days of

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Table 1. Chemical composition of feed ingredient (% on DM basis)

Particular	Concentrate mixture	Lucerne
DM	90.97	17.23
OM	88.15	83.25
CP	19.67	16.90
EE	3.20	1.92
Total ash	7.85	11.20
NDF	40.79	41.30
ADF	13.68	26.60
ADL	4.58	7.68
Zinc	28 ppm	30 ppm
Lead	0.5ppm	0.4ppm

Table 2. Effect of Zn supplementation to Pb exposed goat kids on lymphocyte proliferation

Days	Group 1	Group 2	Group 3	SEM
0	1.031	1.013	1.005	0.020
30	1.235	0.962	1.125	0.009
60	1.847	1.275	1.537	0.007
90	1.647	1.101	1.475	0.009
Mean $\pm$ SE	1.440 ^a $\pm$ 0.186	1.088 ^c $\pm$ 0.068	1.285 ^b $\pm$ 0.130	

Means bearing different superscripts differ significantly ($P < 0.01$).

treatment diets for observing lymphocyte proliferation and SOD expression. The proliferative response of lymphocyte was estimated using the colorimetric MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay (Mosmann 1983). Real time PCR primers were designed to monitor SOD expression. Primers for SOD were (CAC GAC GAG GCA AAG GGA GAT ACA GTC GTG) and reverse primer (TCC AAA CTG ATG GAC GTG GAA) and for β -Actin (GTA CGA TGG GCC AGA AGG ACT CGT AC) and reverse primer (TGACGATGCGTGCTCCAT) (25 nmol, 15–45, 3 ODs). The amplicon product size was 93 bp for Cu/Zn SOD gene, and 96 bp for β -Actin gene. Complementary DNA was synthesized from cultured lymphocyte using a cell to cDNA II kit. Total RNA was isolated from bovine blood leukocytes according to the manufacturer's instructions. Real-time PCR was performed using the SYBR Green qPCR SuperMix as a double-stranded DNA-specific fluorescent dye in 25 μ l reaction to assess the SOD mRNA expression relative to housekeeping gene β -actin. Each cDNA sample was analyzed in triplicate for quantitative assessment of RNA amplification with PCR primers.

Data computation and statistical analysis: Data on lymphocyte proliferation were statistically analyzed (Snedecor and Cochran 1994) by two-way ANOVA using SPSS 17.0. The comparative C_T method was used for relative quantification of SOD mRNA and quantification was normalized to the internal control β -actin gene. Fold changes in relative mRNA expression of the target genes were determined using the formula $2^{-\Delta\Delta C_T}$. Effect of treatments was

analyzed by one-way ANOVA using SPSS 17.0. Differences with probabilities (P) < 0.05 were considered significant. Relative quantification of target gene expression levels as fold-difference was based on three sets of samples. Data on mRNA expression were analyzed using the light cycler software.

RESULTS AND DISCUSSION

Dry matter intake: The weekly dry matter intake (g/day) of goat kids in the 3 groups (Fig 1.) showed no effect of dietary treatments. Arvind Kumar (2003) supplemented 100 ppm Pb for 90 days in the diet of growing calves as well as lactating cows and did not observe any change in their DM intake. However, supplementation at 1000 ppm level in dairy calves led to reduction in feed intake by 9.5% (Neathery *et al.* 1987). Reduced feed intake was also noticed by Longer *et al.* (1984) in dairy calves fed 18 mg Pb/kg b.wt. thrice a week. Since the level of Pb supplementation in the diet of kids in the present study was within the permissible limits and the duration of the experiment was also less, so, no adverse effect of Pb was noticed on the feed intake and therefore, addition of Zn to Pb exposed kids did not result in any improvement.

Lymphocyte proliferation: The mean $\pm$ SE values of lymphocyte proliferation response for all the 3 groups are given in Table 2. Mitogen induced lymphocyte blastogenesis increased as the age of the kids increased in groups 1 and 3, but, this trend was not evident in group 2 probably due to the feeding of Pb to these kids. Overall average lymphocyte proliferation response at the end of 90 days duration was significantly ($P < 0.05$) lower in group 2 (1.088) as compared to groups 1 (1.440) and 3 (1.285) due to Pb supplementation. This adverse effect on lymphocyte proliferation was recovered to some extent by Zn, but, it was still significantly less than the control, indicating that Zn addition in the diet of Pb exposed kids could not fully recover the adverse effect caused by Pb. Protective effect of Zn supplementation against Pb induced toxicity is mainly due to their interactions in several biological and toxicity reactions (Prasanthi *et al.* 2005). Zn also plays an important role in cell-mediated immunity (Meunier *et al.* 2005)

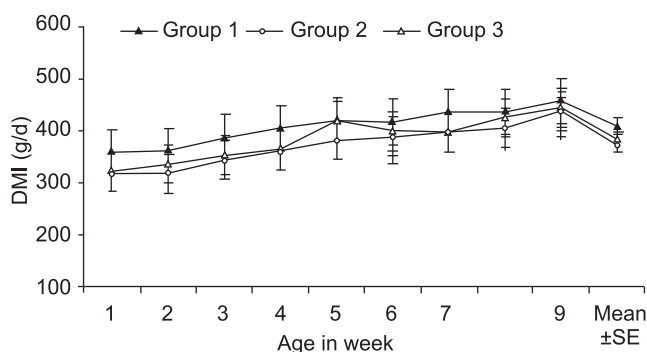


Fig 1. Effect of Zn supplementation to Pb exposed goat kids on dry matter intake (DMI).

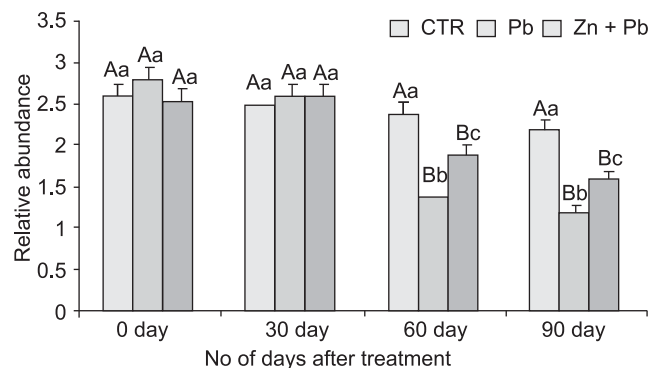


Fig 2. Effect of Zn supplementation to Pb exposed goat kids on SOD expression. (Bars having different letters (A, B, a, b and c) differ significantly ($P < 0.05$) from one another.).

through its involvement in cell replication and proliferation (Weiss and Spears 2006) and even minute alterations in the Zn level influence T cell development as well as T cell functions. Similarly in the present study, the zinc supplemented diet resulted in higher lymphocyte proliferation as compared to lead supplemented diet.

Superoxide dismutase (SOD) gene expression: SOD expression as shown by relative abundance at the start of the experiment was similar in all the different treatments (Fig. 2.). SOD expression remained almost similar in group 1 during 90 days period, but substantial decrease in relative abundance of SOD at 60 days was observed in Pb supplemented group 2 which further decreased at 90 days of Pb exposure. Decrease in SOD activity after varying levels of lead exposure was reported earlier also (Ye *et al.* 1999, Mousa *et al.* 2002, Vaziri *et al.* 2003, Kasperczyk *et al.* 2004, Farmand *et al.* 2005). The decrease in SOD expression was recovered to some extent in goat kids of group 3 supplemented with Zn showing positive effect of this metal. Shay and Cousins (1993) suggested that metallothionein (MT), a protein involved in Zn homeostasis and its mRNA abundance is increased when Zn was supplemented to Zn deficient animals. Jing *et al.* (2007) reported reduction in the Cu-Zn superoxide dismutase (Cu-Zn SOD) and catalase (CAT) activity, and increase in the malondialdehyde and hydrogen peroxide contents in rats fed Zn deficient diet, whereas the rats fed zinc excess diet had higher Cu-Zn SOD activity. It is inferred that Pb supplementation to young goat kids reduced their SOD expression and immunity status, whereas, supplementation of 50 ppm Zn in their diet could ameliorate these adverse effects to some extent.

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