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Original article

Ameliorative effect of zinc supplementation to lead exposed goat kids on immune status

M. Kumar[a,] *, H. Kaur[a], B.T. Phondba[b], G. Chandra[c], R.S. Deka[a], V. Sharma[a]
[a]*Dairy Cattle Nutrition Division, National Dairy Research Institute, Karnal, India.*
[b]*National Dairy Development Board, Anand, India.*
[c]*Dairy Cattle Physiology Division, National Dairy Research Institute, Karnal, India..*

*Corresponding author; Dairy Cattle Nutrition Division, National Dairy Research Institute, Karnal, India, Tel: +91-9045546435.

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A B S T R A C T

In vivo studies were conducted to observe the adverse effects of lead and protective effect of zinc on lymphocyte proliferation and total immunoglobulins (Ig) concentration in eighteen crossbred (Alpine x Beetal) male goat kids. They were divided into three groups i.e. Group I (Control), Group II (Control + 50 ppm Pb) and Group III (Control + 50 ppm Pb + 50 ppm Zn). All the kids were fed as per standard dietary requirements for a period of 90 days**.** Blood samples were collected on 0, 30, 60 and 90 days of Pb and Zn supplementation for lymphocyte separation and total Ig. A fixed no. of cells ($2x10^6$) was grown in culture for 72 hours for studying the lymphocyte proliferation. Overall average lymphocyte proliferation response at the end of 90 days duration was significantly ($P<0.05$) lower in Pb supplemented group II (1.088) as compared to groups I (1.440) and III (1.285). 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. Results revealed significant ($P<0.05$) decrease in the mean Ig concentration (mg/ml) in group II, but it was similar in groups I and III. It may be concluded that supplementation of Zn in the diet of Pb exposed kids had a beneficial effect on lymphocyte proliferation and Ig concentration.

1. Introduction

Heavy metals are recognized as environmental pollutants and are released from both industrial and agricultural sources. Lead is the most common toxic mineral and the most abundant contaminant of environment. Ingestion of lead through contaminated herbages and soil leads to toxicity in animals resulting in heavy mortality. 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 Kueh, 2005). Pb induced accumulation of reactive oxygen species (ROS), reduce the immunity status of the animals by affecting the cell-mediated immunity and neutrophil function. It is well known that 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). 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. Animals deficient in zinc are more susceptible to be poisoned with lead, because there is increased absorption of this mineral element (Alonso *et al.,* 2004). Therefore, this study was conducted to examine the effect of zinc supplementation to lead exposed goat kids on their immunity status.

2. Materials and methods

Eighteen crossbred (Alpine x Beetal) male goat kids were selected from National Dairy Research Institute (NDRI) herd and randomly divided into three groups .Group I was kept as control and groups II and III were administered 50 ppm Pb, and group III 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 (Table 1) and lucerne (*Medicago sativa*) fodder. Chemical composition of feeds offered to goat kids during the experimental period of 90 days is presented in Table 2. 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 three 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 treatment diets for observing lymphocyte proliferation and total plasma Ig. The proliferative response of lymphocyte was estimated using the colorimetric MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay (Mosmann, 1983). Plasma Ig were estimated by zinc turbidity method (Mc Ewan and Fisher, 1970). The results are expressed as means ± SD. The data generated were statistically analyzed by two way ANOVA using SPSS 17.0 statistical package program (SPSS Inc, Chicago, IL, USA), according to Snedecor and Cochran (1994). Statistical significance was set at $p< 0.05$.

3. Results and discussion

3.1. Dry matter intake (DMI)

The weekly dry matter intake (g/day) of goat kids in the three groups is presented in Table 3, showing 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 mgPb/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 for short duration. Therefore no adverse effect of Pb was noticed on the feed intake and addition of Zn to Pb exposed kids did not result in any improvement.

3.2. Lymphocyte proliferation (lymphocyt stimulation index, LSI)

The mitogen induced lymphocyte blastogenesis increased as the age of the kids increased in groups I and III, but, this trend was not evident in group II probably due to feeding of Pb to these kids Table 4. Overall average lymphocyte proliferation response at the end of 90 days duration was significantly (P<0.05) lower in group II (1.088) as compared to groups I (1.440) and III (1.285) due to Pb supplementation.

Table 1
Composition of concentrate mixture.

Sr. no.	Ingredients	Parts
1	Groundnut cake	21
2	Maize	33
3	Wheat bran	20
4	Rice bran	11
5	Deoiled mustard cake	12
6	Mineral mixture	2
7	Common salt	1

Table 2
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 3
Effect of zinc supplementation to Pb exposed goat kids on dry matter intake (g/d).

Week	Group I	Group II	Group III
1	358.62 ± 43.61	319.08 ± 36.86	321.27 ± 36.86
2	360.85 ± 43.61	318.26± 36.86	336.07 ± 36.86
3	386.22 ± 43.61	343.04 ± 36.86	352.87 ± 36.86
4	404.40 ± 43.61	361.94 ± 36.86	363.12 ± 36.86
5	419.57 ± 43.61	381.59 ± 36.86	419.65 ± 36.86
6	417.82 ± 43.61	388.62 ± 36.86	399.29 ± 36.86
7	434.74 ± 43.61	396.49 ± 36.86	397.90 ± 36.86
8	435.53 ± 43.61	405.30 ± 36.86	425.22 ± 36.86
9	456.72 ± 43.61	437.49 ± 36.86	444.64 ± 36.86
Mean ± SE	408.27 ± 14.54	372.42 ± 12.29	384.45 ± 12.29

groupI, groupII and groupIII show no significant difference in weekly DMI.

Table 4
Effect of Zn supplementation to Pb exposed goat kids on lymphocyte proliferation.

Days	Group I	Group II	Group III
0	1.031 ± 0.014	1.013 ± 0.028	1.005 ± 0.019
30	1.235 ± 0.010	0.962 ± 0.009	1.125 ± 0.009
60	1.847 ± 0.004	1.275 ± 0.013	1.537 ± 0.006
90	1.647 ± 0.003	1.101 ± 0.016	1.475 ± 0.008
Mean ± SE	1.440^{a} ± 0.186	1.088^{c} ± 0.068	1.285^{b} ± 0.130

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

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. It might be that the quantity of Zn supplemented to counteract the adverse effect of Pb was not sufficient in this experiment. Lymphocyte stimulation is widely used to measure immune competence by stimulation of lymphocytes with phytomitogens (Weigel et al. 1992). A series of in vitro studies have demonstrated that exposure of bovine peripheral blood mononuclear cells to Pb reduced their responsiveness to mitogens or decreased the number of viable cells (Jakway et al. 1971) similar to the present studies. Zn plays an important role in cell-mediated immunity (Meunier et al. 2005) through its involvement in cell replication and proliferation (Weiss and Spears 2006). Even minute alterations in the Zn level influence T cell development as well as T cell functions. 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).

3.3. Plasma total immunoglobulin (Ig)

It was observed that the total Ig concentration in Pb supplemented group II decreased as the days of treatment increased (Table 5).

Table 5

Effect of Zn supplementation to Pb exposed goat kids on total Ig concentration (mg/ml).

Days	Group I	Group II	Group III
0	29.51 ± 0.81	29.88±0.40	29.47±0.69
30	31.09 ± 0.82	29.29±0.40	30.24±0.36
60	29.79±0.48	28.00±0.46	30.40±0.33
90	31.02±0.84	26.18±0.43	30.54±0.42
Mean ± SE	30.35^a ± 0.40	28.34^b ± 0.81	30.16^a ± 0.23

Those Means bearing different superscripts differ significantly (P<0.05)

Overall average Ig concentration at the end of 90 days was 30.35 ± 0.40, 28.34 ± 0.81 and 30.16 ± 0.23 mg/ml in the three groups, respectively. The Ig concentration was similar in groups I and III, whereas, it was significantly (<0.05) lower in group II showing the adverse effect of Pb which was recovered due to Zn supplementation. Pb is reported to be responsible for reducing circulating antibody or Ig titers (Jakway et al. 1971). Antibody synthesis is decreased Pb inhibiting the activity of B lymphocyte because B-cells are involved in the production of antibodies or Ig. As Pb is responsible for production of ROS and immune cells are particularly sensitive to oxidative stress due to high content of unsaturated fatty acids in immune cell membrane, therefore, this might be responsible for decreased Ig levels in group II.

4. Conclusion

Addition of 50ppm Pb in the diet of male goat kids did not affect their feed intake, but showed adverse effects on immunity. These adverse effects on immunity parameters was recovered to some extent by 50 ppm Zn supplementation, indicating that the amount of Zn addition in the diet of Pb exposed kids may be more to counteract the adverse effect of Pb in goat kids.

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