Journal of
Clinical & Cellular Immunology

Kumar et al., J Clin Cell Immunol 2012, 3:2
DOI: 10.4172/2155-9899.1000119

Research Article Open Access

Ameliorative Effect of Zinc Supplementation to Lead Exposed Goat Kids on Immune Status

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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 total immunoglobulins concentration in eighteen crossbred (Alpine x Beetal) male goat kids (around 6 months of age). 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 immunoglobulin. 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.

Keywords: Goat kids; Lymphocyte proliferation; Immunoglobulins; Oxidative stress; antioxidants

Abbreviations: Pb: Lead; Zn: Zinc; DM: Dry Matter; Ig: Immunoglobulins; OM: Organic Matter; CP: Crude Protein; EE: Ether Extract; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre; ADL: Acid Detergent Lignin

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. It is commonly found in soil especially near roadways, older houses, mining areas, industrial sites, power plants, incinerators and hazardous waste sites due to contamination. 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 [1]. Pb induced accumulation of Reactive Oxygen Species (ROS) such as superoxide (O_2^-) leading to oxidative stress. 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 [2]. 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. Animals deficient in zinc are more susceptible to be poisoned with lead, because there is increased absorption of this mineral element [3]. Therefore, this study was conducted to examine the effect of zinc supplementation to lead exposed goat kids on their immunity status.

Materials and Methods

Eighteen crossbred (Alpine x Beetal) male goat kids (around 6 months of age) were selected from 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 kids were vaccinated against PPR and Eterotoximia in every year. Deworming of kids was done before start of experiments. All the kids were fed as per NRC [4] 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 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 Immunoglobulins. The proliferative response of lymphocyte was estimated using the colorimetric MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay [5]. In procedure given

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Received April 13, 2011; **Accepted** May 15, 2012; **Published** May 22, 2012

Citation: Kumar M, Kaur H, Phondba BT, Mani V, Chandra G, et al. (2012) Ameliorative Effect of Zinc Supplementation to Lead Exposed Goat Kids on Immune Status. J Clin Cell Immunol 3:119. doi:10.4172/2155-9899.1000119

by Mosmann, the lymphocyte suspension was adjusted to $2x10^6$ live lymphocytes/ml by the culture media (DMEM) containing 10% FBS. 10 µl of the diluted cell suspension per well in triplicate was placed in a 96 well flat bottomed tissue culture plate. The mitogen employed in the present study was Concanavalin A (Con A) at the concentration of 5 µg/ml of the final culture volume (250 µl), a concentration that had been determined previously to provide maximal stimulation of lymphocyte. In all the cases, final culture volume was 250 µl. The cells were allowed to proliferate with and without mitogen (Con A) to determine the difference between cell proliferations. The blank wells consisted of 250 µl of culture media only. All cultures were allowed to incubate at 37°C in a humidified CO_2 incubator (95% air and 5% CO2) for 72 h.

Plasma immunoglobins were estimated by zinc turbidity method [6].

Statistical Analysis

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 [7]. Statistical significance was set at $p < 0.05$.

Results

The weekly dry matter intake (g/day) of goat kids in the three groups is presented in Table 2 showing no effect of dietary treatments. 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 3. 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. 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. It was observed that the total immunoglobulin concentration in Pb supplemented group II decreased as the days of treatment increased. Overall average immunoglobulin concentration at the end of 90 days was 30.35 ± 0.40, 28.34 ± 0.81and 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 ($p<0.05$) lower in group II showing the adverse effect of Pb which was recovered due to Zn supplementation.

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

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

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

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

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)

Table 3: Effect of Zn supplementation to Pb exposed goat kids on lymphocyte proliferation.

Discussion

Arvind Kumar [8] 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% [9]. Reduced feed intake was also noticed by Longer et al. [10] in dairy calves fed 18mgPb/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. Feeding of another heavy metal Cd within permissible limits to crossbred male calves upto 120 days also did not cause any depression in their DM intake [11]. Lymphocyte stimulation is widely used to measure immune competence by stimulation of lymphocytes with phytomitogens [12]. 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 [13] similar to the present studies. Zn plays an important role in cell-mediated immunity [14] through its involvement in cell replication and proliferation [15]. Even minute alterations in the Zn level influence T cell development

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)

Table 4: Effect of Zn supplementation to Pb exposed goat kids on total Immunoglobulin concentration (mg/ml).

as well as T cell functions. Bartoskewitz et al. [16] supplemented 1000 ppm Zn and 200 ppm Cu to deers maintained in captivity and observed improved lymphocyte proliferation. Protective effect of Zn supplementation against Pb induced toxicity is mainly due to their interactions in several biological and toxicity reactions [17]. Pb is reported to be responsible for reducing circulating antibody or immunoglobulin titers [13]. Antibody synthesis is decreased by Pb by inhibiting the activity of B lymphocyte because B-cells are involved in the production of antibodies or immunoglobulins. As Pb is responsible for production of ROS and immune cells are particularly sensitive to oxidative stress, therefore, this might be responsible for decreased Ig levels in group II.

Acknowledgements

We are thankful to the National Dairy Research Institute, Karnal and Indian Council of Agricultural Research- Pusa, New Delhi, India for the fellowship award for carrying out this research work. I would also like to acknowledge partial financial assistance by the National Bureau of Animal Genetic Research, Karnal, India.

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