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AGR2015-1773

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This paper should be cited as follows:

Varadan, D., Vazhoor, B., Dass, R. S. and Garg, A. K. (2016). **"Ameliorative Effect of Dietary Zinc and Rumen Protected DL-Methionine on Nitrogen, Calcium and Lead Retention Alterations Induced by Lead Toxicity in Goats",** Athens: ATINER'S Conference Paper Series, No: **AGR2015-1773.**

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URL Conference Papers Series: www.atiner.gr/papers.htm

ISSN: 2241-2891
05/01/2016

Ameliorative Effect of Dietary Zinc and Rumen Protected DL-Methionine on Nitrogen, Calcium and Lead Retention Alterations Induced by Lead Toxicity in Goats

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Abstract

The present experiment was aimed to assess the ameliorative effect of dietary zinc and DL-methionine in goat kids exposed to 250 ppm lead for 150 days. Thirty healthy male goat kids of about 3-4 months of age were divided into five groups of six animals in each, on the basis of their body weight following a randomized block design. While the control group (G1) was fed with a basal diet, the treatment groups were supplemented with 250 ppm Pb as lead acetate (G2); 250 ppm Pb + 250 ppm zinc (G3); 250 ppm Pb + 250ppm rumen protected DL-methionine (G4); and 250 ppm Pb + 250ppm Zn + 250 ppm rumen protected DL-methionine (G4) Experimental feeding lasted for a period of 150 days. Dietary lead supplementation had a significant effect on feed intake and the ether extract digestibility, plane of nutrition and growth. The highest nitrogen balance ($P<0.05$) was noted in group G1 and the lowest ($P<0.05$) in G2. Calcium (Ca) excretion through faeces was significantly ($P<0.05$) higher in all the lead supplemented groups and comparatively lesser Ca excretion was noted in group G4 where 250 ppm rumen protected DL-methionine was supplemented along with 250 ppm Pb in their diets. The results indicated the negative interaction between Pb and Ca at the absorption sites and the protective effect of 250 ppm rumen protected DL-methionine, probably by more chelation of lead at the absorption sites. The retention of Pb (mg/d) was significantly ($P<0.05$) higher in the treatment groups compared to the control group. Retention was significantly ($P<0.05$) reduced in group G4 when compared to G2, G3 and G5, indicating the ameliorative effect of 250 ppm rumen protected DL-methionine added in the diet.

Keywords: Dietary amelioration, DL-methionine, Goat kids, Lead, Retention

Acknowledgments: The authors are thankful to the Director, IVRI for providing facilities to carry out the work and for providing an institutional scholarship to the first author for carrying out his Ph.D programme.

Introduction

Lead has been incriminated as one of the most common causes of accidental poisoning in domestic and wild animals, as well as humans. There are several reports documenting lead toxicity in livestock (Dey et al., 1996; Swarup et al., 2005). In the recent years, the lead consumption in India was around 2.5 lakh tons, out of which about 75 percent is being used by the lead-acid battery sector. Almost all automobile batteries contain lead and it is also used in underground cable sheath, alloys and pigments. Lead is used in batteries of UPS and power inverters and its business is increasing day by day.

The atomic symbol for lead is Pb, which derives from Latin word *plumbum*. Lead is a bluish to silvery grey heavy metal, which is soft, pliable and has no characteristic taste or smell. Lead is not known to be an essential nutrient for animals and does not participate in any known beneficial biochemical functions (NRC, 2005). The environmental health of developing countries like India and most of the underdeveloped countries is in a state of disaster mostly due to industrial emission and automobile smoke in and around urban and sub-urban areas, risking the human and animal population to the potential exposure of environmental lead (WHO, 1989). Anthropogenic activities such as mining, smelting and use of automobile fuels have substantially altered the natural ecological distribution of lead in the environment leading to globally elevated levels of lead in air, water and soil (Nriagu, 2009). Environmental pollution leading to animal feed contamination and thereby livestock getting exposed to higher levels of lead is now a common feature in developing countries. The maximum tolerable level for ruminants is 250 mg lead/kg diet (NRC, 2005).

Various conventional chelators such as calcium versenate (calcium disodium EDTA) and di-mercapto succinic acid (DMSA) have been used to ameliorate the toxicity of lead, but various side effects such as nephrotoxicity have been observed (Blood et al., 1983). Zinc is being proposed as an ameliorating agent since both zinc and lead compete for the same absorptive and enzymatic sites and might decrease the absorption of lead (Flora et al., 1982).

Lead has a high affinity for glutathione (GSH) and lead-GSH complex gets excreted through urine. This depletes the cells of their GSH and decreases the antioxidant capacity of the animal. Methionine/cysteine when supplemented in diet can provide the sulfhydryl groups for glutathione formation (Dildeep et al., 2013a; 2013b). There is little information on the use of zinc and methionine as ameliorative agents against lead toxicity in animals. Therefore, the present study aims to study the ameliorative effects of zinc and rumen protected DL-methionine on the adverse effects of lead in goats.

Materials and Methods

Experimental Animals and Feeding

Thirty healthy male kids of about 2-3 months of age were obtained from the Sheep and Goat Farm of IVRI, Izatnagar. These animals were maintained for 1 month on a standard diet comprised of a concentrate mixture and oat straw, before the start of the proper experiment. The kids were housed in Animal Nutrition Division Sheds, having facilities for individual feeding and watering. The kids were vaccinated against peste des petits ruminants (PPR), Black quarter (BQ) and Haemorrhagic septicemia (HS) adopting standard protocol. After adapting the kids for one month, they were divided into six blocks of five animals in each on the basis of body weight in such a way that within each block animals are homogenous. Then five treatments were allocated randomly to animals in each block separately. Thus the design adopted was RBD.

Housing and Management

All the experimental kids were housed in a well ventilated shed with provision of individual feeding and watering. Strict management and hygienic practices were adopted throughout the experimental period. All the kids were dewormed against ecto and endo parasites before the start of the experiment and subsequently at regular intervals. Clean drinking water was provided *ad libitum* twice a day at about 10 A.M. and 3 P.M. daily**.**

Feeds and Feeding

The goats were offered a concentrate mixture and oat straw to meet their nutrient requirements as per NRC (2007) recommendation for a body weight gain of 50g/day. The concentrate mixture consisted of maize (35%), soybean meal (30%), wheat bran (32%), mineral mixture (2%) and common salt (1%). A weighed amount of concentrate mixture was provided at 9.30 A.M daily to meet almost their whole crude protein (CP) and the major part of their total digestible nutrient (TDN) requirements. Oat straw was provided *ad libitum* after complete consumption of the concentrate mixture. The amount of concentrate mixture required by each goat was adjusted every fifteen days based on their body weights. All the kids were provided with 100 g berseem hay, twice a week to meet their vitamin A requirements. Experimental feeding lasted for 150 days including a 6 day metabolic trial period**.** The different dietary treatments are given in Table 1 below.

Table 1. *Distribution of Animals to Different Dietary Treatments*

Group	**Treatment**
G1(control)	Basal diet
G2	Basal diet + 250 ppm Pb
G3	Basal diet + 250 ppm Pb + 250 ppm Zn
G4	Basal diet + 250 ppm Pb + 250 ppm methionine **(in protected form)**
G5	Basal diet + 250 ppm Pb + 250 ppm Zn + 250 ppm methionine **(in protected form)**

Lead was supplemented in the diet as lead acetate, zinc as zinc sulphate and methionine as protected methionine (METIPEARL DRY, Kemin Industries South-Asia Pvt Ltd).

Recording of Body Weights and DM Intake

Body weights of all the animals were recorded at fifteen day intervals during morning time before offering them any food and water. Animals were offered a weighed amount of concentrate mixture and oat straw. Residue, if any was weighed after 24 h of feeding. Samples of concentrate mixture and oat straw were subjected to dry matter (DM) analysis to know their daily DM intake.

Metabolism Trial

To determine the nutrient digestibility, plane of nutrition and balances of N, Ca, P, Pb, Zn and Cu, a metabolism trial of a 6 day collection period was carried out after 100 days of experimental feeding. Four animals from each group were randomly selected and kept in metabolic cages, which were having facilities for separate urine and faeces collection. After adapting the animals in metabolic cages for 3 days, the actual collection of faeces and urine started

The animals were fed individually with a weighed amount of feed. Residue left and the total faeces voided by the individual animals were collected after 24 h, weighed and a representative sample of the residue and faeces was brought to the laboratory for further analysis. An aliquot of feed, residues and faeces were kept in a hot air oven at 100 ± 1^{0}C to estimate the DM content. Samples of 6 days were pooled together and stored in separate plastic sachets for each individual animal for further analysis. A suitable aliquot of faeces was transferred daily to a Kjeldahl flask containing 25 ml of commercial sulphuric acid for nitrogen estimation. Suitable aliquots of urine were taken in duplicate and pooled daily into Kjeldahl flask containing 40 ml commercial sulphuric acid for nitrogen estimation. Another appropriate aliquot was collected into 100 ml plastic bottles and pooled samples were refrigerated for analysis of the minerals.

Chemical Analysis

Feed, fodder, residue and faeces samples were analyzed for proximate principles as per AOAC (2005). Lead, zinc and copper in feed, fodder and feces were estimated by atomic absorption spectrophotometry (AOAC, 2005), while the calcium and phosphorus content was estimated by spectrophotometry following standard protocols.

Statistical Analysis

All the data generated were analysed statistically by using SPSS software (version 10.1.4). Data were analysed by using a generalised linear model, univariate ANOVA procedure (Snedecor and Cochran, 1994). If the F-value of the treatment group was found to be significant, the Duncan Multiple Range test (DMRT) was adopted as a post hoc analysis for the comparison of treatment groups.

Results and Discussion

There was a significant (P>0.05) reduction in the final body weight, weight gain and average daily gain in groups, G2 and G5 (Table 2). The feed: gain ratio was also increased in groups, G2 and G5 when compared to other groups. These results showed the growth depressing effect of 250 ppm lead in the diet of goat kids. 250 ppm zinc and 250 ppm rumen protected DL-methionine given to kids of group G3 and G4 provided a significant ameliorative effect on growth, while the 250 ppm zinc + 250 ppm rumen protected DL- methionine used in group G5 was not as effective as 250 ppm zinc or 250 ppm rumen protected DL- methionine used alone, probably due to the frequent loose motion encountered in kids of group G5. The protective effect of methionine on lead toxicity at the growth level was previously reported by Shehata (2011), Sharma et al. (2009) and Latta and Donaldson (1986). They postulated that lead depresses the glutathione levels and methionine supplemented favours the production of glutathione and the Pb - glutathione complex gets excreted, reducing the effect of lead on growth.

Table 2. *Mean Body Weight (Kg) of Kids in Different Groups at 15 Days Interval*

Days	Group					SEM	P value
	G1	G2	G3	G4	G5		
Initial Body Weight(0 day)	8.3	8.3	8.4	8.4	8.3	0.28	0.998
15	8.0	8.1	8.7	9.1	8.4	0.28	0.741
30	8.6	8.3	9.3	9.4	8.1	0.32	0.631
45	8.9	8.7	9.8	9.8	8.6	0.39	0.485
60	9.9	8.9	10.8	10.4	9.1	0.42	0.281
75	10.3	9.1	11.1	11.0	9.7	0.43	0.166
90	11.0	9.8	11.8	11.6	10.3	0.40	0.174
105	12.0	10.4	12.1	12.1	10.9	0.39	0.208
120	12.5	10.3	12.2	12.3	11.4	0.40	0.276
135	13.0	10.4	12.7	12.7	11.6	0.39	0.145
150 *	13.6^{a}	10.9^{c}	13.3^{a}	13.4^{a}	11.9^{b}	0.39	0.049

*Means bearing different superscripts in a row differ significantly (P< 0.05)

Similarly, the protective effect of zinc against lead toxicity has been previously reported by many other workers including Cerklewski and Forbes (1976 b), Flora et al. (1982) and Batra et al. (2001). They postulated that Zn is a hepatic metallothionein inducer and it competitively reduces the intestinal absorption of lead, further Zn is a component of antioxidant enzymes.

On the contrary, no adverse effect on the growth rate and feed efficiency was noted by Logner et al. (1984) in calves supplemented with lead sulfate at 500 mg/kg diet. However, a 1000 mg lead sulfate /kg diet resulted in decreased weight gain and feed efficiency in calves (Neathery et al., 1987).

There was statistically no significant (P>0.05) difference between the groups in the total dry matter intake (DMI) and in average DMI due to supplementation of 250 ppm of dietary Pb. The growth reduction in group G2

and G5 could be attributed to the metabolic changes brought about by the dietary lead supplementation. There was a statistically ($P<0.05$) significant reduction in ether extract digestibility in all the lead supplemented groups when compared to control group G1. This could probably be due to the interaction between lead and long chain fatty acids in the rumen. The results corroborated well with the findings of Kumar and Chopra (2003) who reported a reduced ether extract digestibility in calves fed diets containing 50 and 100 ppm lead. The statistical analysis of the data on CPI (g/kg $W^{0.75}$), DCPI (g/kg$W^{0.75}$), TDNI (g/d) and TDNI (g/kg$W^{0.75}$) did not reveal any significant differences ($P>0.05$) when compared among the different groups.

The nitrogen excretion through faeces was significantly ($P<0.05$) higher in group G2 and G5 when compared to group G1, G3 and G4 (Table 3). The shift in more excretion of nitrogen through faeces in group G5 could be attributed to the frequent diarrhoea observed in G5 kids. The total nitrogen outgo was the highest ($P<0.05$) in group G2 followed by G5 and it was comparable among group G1, G3 and G4. The highest nitrogen balance ($P<0.05$) was noted in group G1 and the lowest ($P<0.05$) in G2. Nitrogen excretion through urine in group G5 was significantly ($P<0.05$) lower when compared to other groups.

Table 3. *Intake and Balance (g/d) of Nitrogen, Calcium and Phosphorus in Different Groups of Kids*

Attribute	Group					SEM	P value
	G1	G2	G3	G4	G5		
Nitrogen							
Intake*	9.15^{a}	8.79^{b}	8.95^{ab}	8.96^{ab}	8.86^{b}	0.04	0.045
Outgo through							
Faeces*	3.43^{c}	3.97^{b}	3.53^{c}	3.36^{c}	4.47^{a}	0.11	0.000
Urine*	2.69^{b}	3.33^{a}	2.68^{b}	2.77^{b}	2.18^{c}	0.09	0.000
Total outgo*	6.12^{c}	7.30^{a}	6.21^{c}	6.13^{c}	6.65^{b}	0.11	0.000
Balance*	3.03^{a}	1.49^{e}	2.74^{c}	2.83^{b}	2.21^{d}	0.13	0.000
As % intake*	33.1^{a}	16.9^{d}	30.6^{c}	31.6^{b}	24.9^{c}	1.36	0.000
Calcium							
Intake	4.23	4.05	4.20	4.18	4.14	0.05	0.767
Outgo through							
Faeces	1.39^{c}	2.00^{a}	2.05^{a}	1.75^{b}	2.00^{a}	0.08	0.024
Urine	0.39	0.40	0.38	0.37	0.39	0.01	0.939
Total outgo	1.78^{b}	2.40^{a}	2.43^{a}	2.12^{ab}	2.39^{a}	0.08	0.025
Balance	2.45^{a}	1.65^{c}	1.77^{c}	2.06^{b}	1.75^{c}	0.08	0.000
As % intake	57.9^{a}	40.7^{c}	42.1^{c}	49.3^{b}	42.3^{c}	1.77	0.000
Phosphorus							
Intake	2.18	2.13	2.19	2.15	2.16	0.02	0.931
Outgo through							
Faeces	0.99	0.99	1.03	0.87	1.00	0.03	0.569
Urine	0.19	0.19	0.19	0.19	0.19	0.00	0.975
Total outgo	1.18	1.18	1.22	1.06	1.19	0.03	0.595
Balance	1.00	0.95	0.97	1.09	0.97	0.02	0.204
As % intake	45.8	44.6	44.3	50.7	44.9	1.17	0.202

*Means bearing different superscripts in a row differ significantly ($P< 0.05$)

The results of the present experiment are contrary to the findings of Fick et al. (1976) who reported no effect on the nitrogen balance in wether lambs fed up to 1000 mg Pb/kg feed for 84 days. Similarly, Kumar and Chopra (2003) observed no effect of 50 and 100 ppm lead acetate in feed on nitrogen intake, fecal and urinary N excretion and N balance in crossbred calves.

The statistical analysis of the data regarding Ca intake revealed no significant differences among the groups. Ca excretion through faeces was significantly ($P<0.05$) higher in all the lead supplemented groups and a comparatively lesser Ca excretion was noted in group G4 where 250 ppm rumen protected DL-methionine was supplemented along with 250 ppm Pb in their diets. The total Ca outgo also followed a similar trend as there was not much variation in Ca excretion through urine between the groups. There was a significant lower Ca balance in all the Pb supplemented groups. Group G4 in which 250 ppm protected DL-methionine was supplemented along with 250 ppm Pb in their diet had a comparatively higher Ca balance when compared to all other Pb supplemented groups viz, G2, G3 and G5. These results indicated the negative interaction between lead and calcium at the absorption sites and the protective effect of 250 ppm rumen protected DL-methionine, probably by more chelation of lead at the absorption sites. Similar antagonism between lead and calcium was observed by Pearl et al. (1983) in sheep.

Results revealed no statistical significant ($P>0.05$) difference in phosphorus intake, its outgo through faeces, urine, total outgo and phosphorus balance, when compared among different groups. Similar to our results, Kumar and Chopra (2003) also did not find any effect of lead supplementation on the intake, excretion and balance of P in crossbred calves.

The mean lead (Pb) intake (mg/d) was statistically ($P<0.05$) higher in the lead supplemented groups (G2, G3, G4 and G5) as compared to the control group (G1) (Table 4). Lead excretion through faeces (mg/d) was statistically ($P<0.05$) higher in the Pb supplemented groups (G2, G3, G4 and G5) as compared to the control group (G1). Among the lead supplemented groups, G4 in which 250 ppm rumen protected DL-methionine was supplemented along with 250 ppm Pb in their diet had the highest lead excretion through faeces when compared to group G2, G3 and G5. Excretion of Pb through urine (mg/d) also followed a similar trend and was statistically ($P<0.05$) different in the 5 groups. It was statistically ($P<0.05$) higher in group G2, G3, G4 and G5 as compared to group G1, whereas G4, in which 250 ppm rumen protected DL-methionine was supplemented along with 250 ppm Pb in their diet, had the highest lead excretion through urine when compared to group G2, G3 and G5. The total excretion of lead through faeces and urine (mg/d) was significantly ($P<0.05$) higher in Pb supplemented groups as compared to the control group. The retention of Pb (mg/d) was significantly ($P<0.05$) higher in the treatment groups, as compared to the control group. Retention was significantly ($P<0.05$) reduced in group G4 when compared to G2, G3 and G5, indicating the ameliorative effect of 250 ppm rumen protected DL-methionine added in the diet. Our results are supported by Quarterman et al. (1980) and Senapati et al. (2001) who have also reported that sulphur containing amino acids increased the urinary excretion of lead and hence can be used as an ameliorative agent

against chronic lead toxicity. The results of the present study suggested that dietary supplementation of 250 ppm lead had a significant effect on intake and ether extract digestibility, plane of nutrition and growth. Nitrogen and calcium balances were significantly affected by lead in their diet. Higher lead retention was observed in all treatment groups. Significant reduction in lead retention observed in rumen protected DL-methionine supplemented groups indicated the ameliorating potential of this dietary amino acid supplement.

Table 4. *Intake and Balance of Lead (mg/d) in Different Groups of Kids*

Attribute	Group						
	G1	G2	G3	G4	G5	SEM	P value
Lead							
Intake*	0.66^{b}	66.37^{a}	64.98^{a}	67.17^{a}	66.06^{a}	5.27	0.000
Outgo through							
Faeces*	0.19^{c}	20.63^{b}	19.82^{b}	26.77^{a}	20.70^{b}	2.04	0.000
Urine*	0.01^{c}	2.27^{b}	2.02^{b}	3.18^{a}	2.14^{b}	0.23	0.000
Total outgo*	0.20^{c}	22.90^{b}	21.84^{b}	29.95^{a}	22.84^{b}	2.25	0.000
Balance*	0.46^{c}	43.47^{a}	43.14^{a}	37.22^{b}	43.22^{a}	3.26	0.000
As % intake*	69.7^{a}	65.5^{a}	66.4^{a}	55.4^{b}	65.4^{a}	2.02	0.000

Conclusions

Results revealed the growth depressing effect of 250 ppm lead in the diet of goat kids. Nitrogen, calcium and lead balances were significantly affected by lead in the diet. Reduced lead retention in rumen protected the DL-methionine supplemented group indicated its ameliorating potential.

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