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Effects of Excess Dietary Selenite on Lead Toxicity in Sheep

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ABSTRACT

The hypothesis that excess dietary selenite ameliorates lead (Pb) toxicosis in domestic sheep was tested. Twenty 6–8-yr-old ewes fed alfalfa pellets were assigned to the following treatments: (1) control; (2) 9.8 mg Pb/kg body weight (b.w.)/d as $PbCO_3$; (3) 3 mg Se/animal/d as $Na_2SeO_3 \cdot 5H_2O$; or (4) a combination of treatments 2 and 3. The gelatin-encapsulated salts were given orally. The study was terminated on d 104, by which time three animals in the Pb group and all five animals in the Pb + Se group had died. All remaining animals were slaughtered on d 104. Lead and Se concentrations were determined in six biweekly-collected blood samples and in soft tissues and bone. Sheep on the control and Se treatments had similar feed intakes, body weights, and tissue Pb levels. Those in the Pb + Se group had lower feed intake, but higher blood Pb values compared with the Pb group. Feeding either element increased ($P < 0.05$) the concentration of that element in blood, kidney, liver, spleen, and bone. Muscle-Pb concentrations were not affected ($P < 0.05$) by treatment. Selenium concentrations in kidney, liver, and muscle were greater ($P < 0.05$), whereas those in heart were less ($P < 0.05$) for the Pb + Se group than for the Se Group. Clinical signs associated with Pb toxicosis noted in other animals were not observed in the poisoned sheep in this study. Selenite did not protect sheep against Pb toxicity and likely served as a synergistic factor.

Index Entries: Alfalfa pellets; blood; bone; kidney; lead; liver; muscle; ovine; selenium; sheep; spleen.

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INTRODUCTION

The toxicity of lead (Pb) to animals may be reduced by the presence of other minerals, especially calcium (Ca) in the diet (*1*). Selenium, as sodium selenite, was mildly protective against the toxic effects of Pb, as the acetate, but only up to 0.5 ppm Se (*2*). At higher sodium selenite levels, a synergistic effect of lead acetate toxicity was observed.

Others (*4*) have shown that lead (5 and 25 ppm) as the acetate antagonizes selenium (1 ppm) in the organic forms in which it occurs normally in foods; low levels of lead (5 ppm) in this case produced high mortality among female mice thus exposed. The following study was initiated to determine whether a high, but not lethal, level of dietary selenite was protective against Pb toxicosis in domestic sheep. These results provided evidence that this level of selenite-Se increased rather than decreased Pb toxicosis in sheep under the experimental conditions employed.

METHODS

Mixed breed ewes, 6–8 yr old and having an average body weight of 63 kg, were allotted by weight into four treatment groups of five sheep each. Treatments were as follows: (1) control, no supplements; (2) daily supplements of 9.8 mg Pb/kg body wt (b.w.)/d; (3) 3 mg Se/animal/d; (4) 9.8 mg Pb/kg b.w./d plus 3 mg Se/animal/d. Lead as $PbCO_3$ and Se as $Na_2SeO_3 \cdot 5H_2O$ were administered orally in gelatin capsules immediately before feeding. Sheep in group 1 did not receive a placebo capsule. Each animal was weighed weekly and the amount of Pb administered to sheep in groups 2 and 3 was adjusted weekly. Lead levels were chosen to produce acute toxicosis in sheep within 6–10 weeks, whereas the Se levels were chosen to be high, but not lethal (*1*). The study was to continue until all sheep in any given treatment group had died.

All sheep had free access to water and NaCl and were group fed, by treatment, alfalfa pellets (1.6 kg/animal/d) that had the following approximate dry matter analysis: 25 mg N/g, 25 mg K/g, 2.7 mg Mg/g, 20 mg Ca/g, 1.6 mg P/g, 600 μg Na/g, 30 μg Mn/g, 200 μg Fe/g, 9 μg Cu/g, 20 μg Zn/g, 5.4 μg Pb/g, and 0.4 μg Se/g. Feed was offered once daily and orts were weighed periodically to calculate feed intake.

Blood was collected at the beginning of the experiment and at about 2-wk intervals thereafter for 64 d. Samples of liver, kidney, spleen, heart, bone, and skeletal muscle were obtained from the sheep that died during the study and from the remaining sheep when they were slaughtered on d 104.

Whole blood, homogenized soft tissues, and defatted bone were freeze-dried. Selenium was determined fluorometrically (*6*) and Pb was determined by flameless atomic absorption using the method of standard

additions (*7*). A national Bureau of Standards (NBS) bovine liver sample was analyzed with each set of Pb samples. The overall mean of the NBS sample was 0.38 ± 0.02 μg Pb/g compared with the certified value of 0.34 ± 0.08 μg Pb/g. Recovery of 0.10 μg Pb additions to tissue samples prior to digestion ranged from 92 to 110 percent. Copper, iron, and zinc in freeze-dried whole blood were determined by flame atomic absorption after digestion in $HNO_3/HClO_4$.

Whole blood was analyzed for urea nitrogen (BUN) and serum was analyzed for Ca, glucose, α-amylase, aspartate aminotransferase (AST), glutamate pyruvate transaminase (SGPT), lactate dehydrogenase (LDH), and creatine kinase (CK) using a Clinicard Analyzer, model 368. Serum inorganic phosphorus was analyzed by a colorimetric phosphomolybdate procedure (*8*). Whole blood was characterized for red cells (RBC), white cells (WBC), neutrophils, lymphocytes, monocytes, eosinophils, and hemoglobin (Hb) by the Hycell 300 cell counter. Blood hematocrit was also determined.

Mineral data for soft tissues and bone were subjected to least squares analysis of variance followed by Duncan's new multiple range test to ascertain differences among treatments (*9*). Blood chemistry data were analyzed by linear regression techniques, without transformation, and the difference between regression coefficients (rate of change = *b*) was evaluated by the *t*-test (*10*).

RESULTS

Feed Intake

Sheep on the control and Se treatments readily ate the 8.0 kg feed (1.6 kg/animal/d) offered daily to each group. The Se-supplemented animals always appeared hungry and fought aggressively for the feed. Feed consumption by animals in the Pb and Pb + Se groups varied throughout the study (Fig. 1). After d 57, sheep on the Pb + Se treatment showed considerable inappetence.

Body Weight Changes

The mean body weight of the sheep at the beginning of the experiment was 63 kg (Fig. 2). During the first 30 d of the experiment, before any sheep died, the control and Se-treated sheep maintained their weight, whereas the Pb treated and Pb + Se treated sheep lost an average of 0.17 kg/animal/d. After d 50, sheep on the control and Se treatments gained 0.12 kg/animal/d for the remainder of the study. Two sheep on the Pb treatment gained up to 3 kg during the remaining period, but these gains were generally offset by weight losses of other sheep in the group. The animal in the Pb + Se group that died on d 104

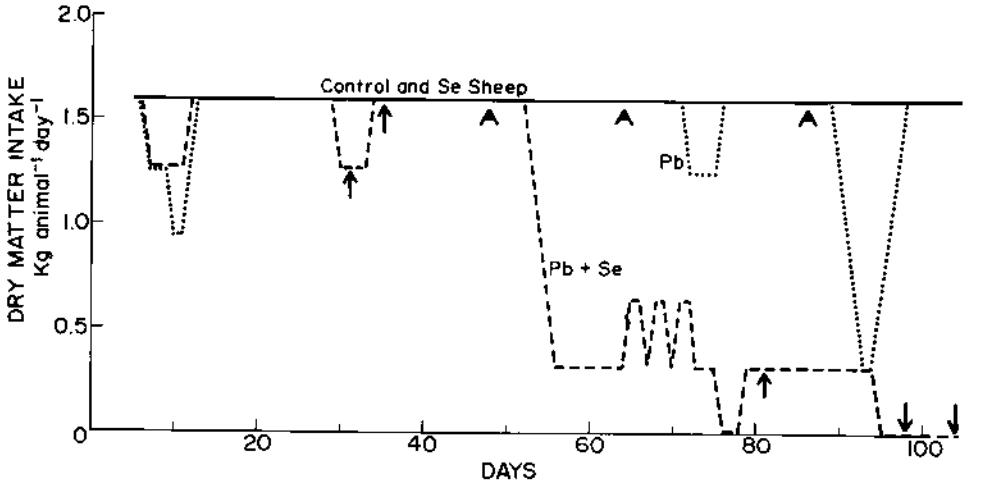

Fig. 1. Running 5-d mean dry matter intake by sheep. Death of sheep is indicated by a chevron (Pb group) or an arrow (Pb + Se).

had a net gain of 2 kg b.w. for the entire experimental period, but the other four animals lost weight from the beginning of the study until they died. Bodyweight changes were not related to body weights at the beginning of the study.

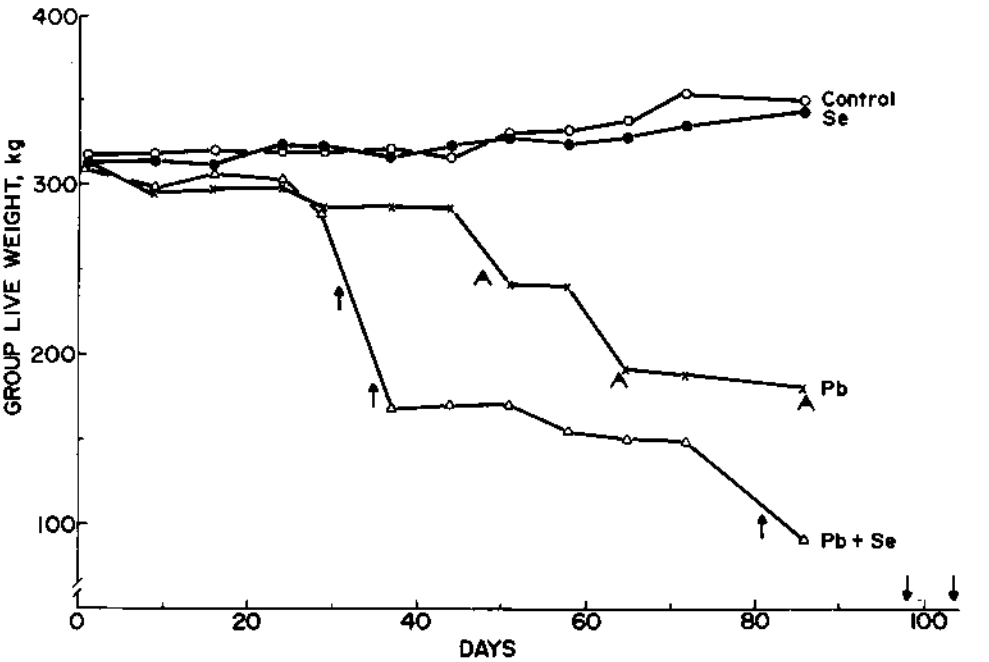

Fig. 2. Group live weight of sheep. Death of sheep in indicated by a chevron (Pb group) or an arrow (Pb + Se).

Death Losses

None of the animals in the control or the Se groups died during the experiment. However, three sheep in the Pb group and all five animals in the Pb + Se group died during the 104 d study (Figs. 1 and 2).

Blood and Tissue Se

Whole blood Se concentrations (Fig. 3) increased 4.2, 7.5, 7.9, and 0 ng/g/d for sheep on the control, Se, Pb + Se, and Pb treatments, respectively. The rates for the Se and Pb + Se groups were not different ($P < 0.2$) from each other, but were greater ($P < 0.01$) than that for the control group, which in turn was greater ($P < 0.06$) than that for the Pb treatment. Blood Se values at the beginning of the experiment were greater ($P = 0.06$) for animals assigned to the control and Se treatments than for those assigned to the Pb and Pb + Se treatments. Although these initial differences may be real, they do not negate the rate of change occurring in response to diet.

Selenium concentrations in kidney, liver, spleen, and bone were greater ($P < 0.05$) in animals receiving the Pb + Se and Se treatments than for those on the control or Pb treatments (Fig. 4). The Pb treatment, reduced ($P < 0.05$) Se concentrations in spleen and heart when compared with similar tissues in the control group. The Pb + Se group had higher ($P < 0.05$) concentrations of Se in kidney, liver, and muscle and lower ($P < 0.05$) concentrations in heart when contrasted with similar tissue concentrations in the Se treatment.

The Se concentrations in tissues of the eight animals dying during the experiment were regressed on the number of days on trial prior to death. The only significant ($P < 0.05$) changes were Se increases in kidney, spleen and heart of 1.71, 0.0067, and 0.0023 μg/g/d, respectively, for

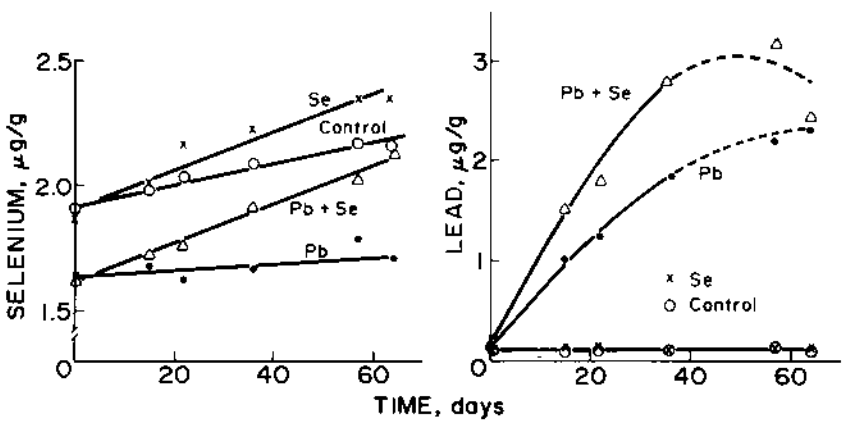

Fig. 3. Mean Se and Pb concentrations in freeze-dried whole blood of sheep.

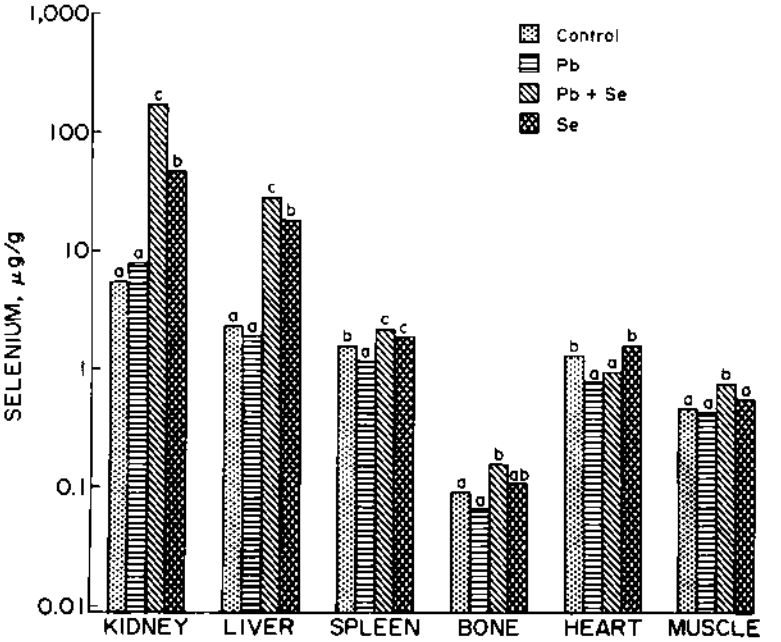

Fig. 4. Mean Se concentrations in freeze-dried tissues and defatted bone of sheep. Treatment Se concentrations, within a given tissue, that do not share a small letter are different ($P < 0.05$).

animals in the Pb + Se group. The 95% confidence intervals (0.95 CI) for these regression coefficients were 0.35, 0.00001, and 0.000004 µg Se/g/d, respectively.

Blood and Tissue Pb

Lead concentrations in whole blood did not differ between the control and Se groups (Fig. 3). The Pb concentrations averaged 0.1 µg/g and did not change during the 64-d sampling period.

However, in the Pb and Pb + Se groups, blood Pb values increased linearly at a rate of 0.05 and 0.09 µg/g/d during the first 37 d, respectively. The rate of change was greater ($P < 0.01$) for the Pb + Se group than for the Pb group. The additive effect of the Se in the Pb + Se diet continued through d 57 ($P < 0.03$), but was not different than the Pb only group on d 64. Blood Pb values for the Pb + Se group and even the Pb group appear curvilinear, but regression of the quadratic ($a + bt + ct^2$) form did not improve the correlation in either case. The apparent curvilinear response of blood Pb values in the two Pb groups may have been confirmed if sampling had continued beyond 64 d and sheep numbers were maintained.

Lead concentrations in kidney, liver, spleen, and bone were not different between the Pb and Pb + Se groups (Fig. 5), but values were

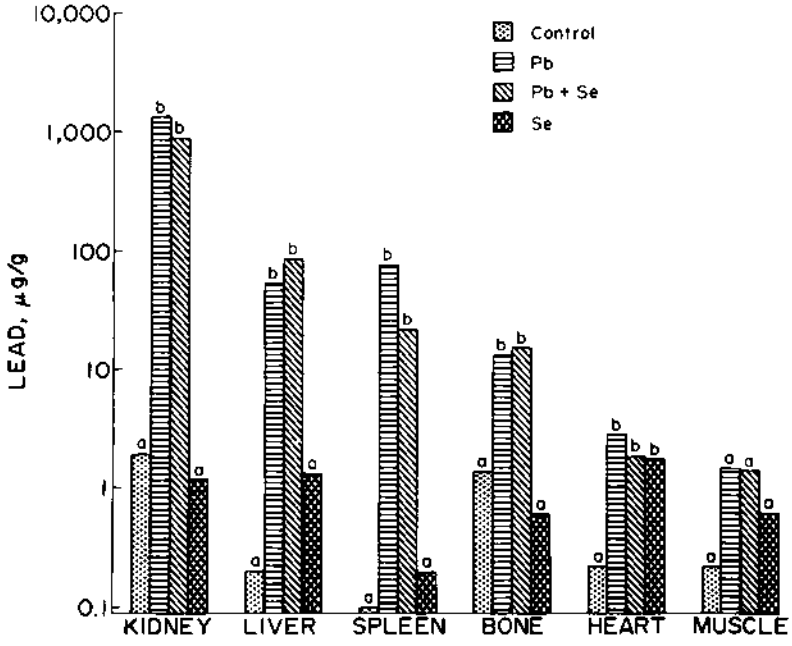

Fig. 5. Mean Pb concentrations in freeze-dried tissue and defatted bone of sheep. Lead concentrations, within a given tissue, that do not share a small letter are different ($P < 0.05$).

larger ($P < 0.05$) than those measured in the non-Pb groups. Muscle-Pb concentrations were not different ($P < 0.05$) for the treatments evaluated in this study. Periodic deaths of three sheep in the Pb and five sheep in the Pb + Se group provided an assessment of Pb accumulation with time. Bone-Pb concentrations increased 0.26 µg/g/d (0.95 Cl = 0.0003) in these eight sheep. Lead accumulations in the soft tissue were not time-related.

Blood Chemistry

Blood chemistry values for each of the treatment groups were regressed against time. Only three of these relationships were significantly different from zero ($P < 0.1$). The Pb treatment increased the serum AST values by 3.8 U/d (0.95 Cl = 2.5) and the serum LDH values by 9 U/d (0.95 Cl = 11). These two enzymes move easily from body tissue and increased serum levels are generally not tissue specific. The Pb + Se treatment increased the serum amylase values by 1.0 U/d (0.95 Cl = 0.025). Elevated levels of these enzymes are often indicative of liver, kidney, pancreas, and muscle damage (*11*). Mean blood chemistry values are shown (Table 1), except that values for treatment responses of serum AST and LDH in the Pb-treatment group and amylase values in the Pb + Se treatment group were omitted.

TABLE 1
Mean Concentration of Constituents in Sheep Blood[a]

Constituent	Mean[a] ± standard deviation
Calcium (S), mg/dL	9.9 ± 0.9
Copper (FDB), μg/g	4.4 ± 0.7
Iron (FDB), mg/g	2.4 ± 0.5
Phosphorus (S), mg/dL	5.4 ± 1.7
Zinc (FDB) μg/g	16 ± 1.7
BUN (B), mg/dL	20 ± 4
Glucose (S), mg/dL	72 ± 8
Amylase (S), IU/L[b]	88 ± 37
SAST (S), IU/L[c]	115 ± 15
SGPT (S), IU/L	14 ± 5
LDH (S), IU/L[c]	360 ± 260
CK (S), IU/L	13 ± 7
Hemoglobin, g/dL	12.8 ± 1.5
RBC × 10^5/mm^3	55 ± 10
WBC × 10^2/mm^3	72 ± 22
Hematocrit, %	39 ± 4
Neutrophils, %	49 ± 14
Lymphocytes, %	44 ± 13
Monocytes, %	4.2 ± 2
Eosinophils, %	3.5 ± 3

[a]Values are for constituents in (S) serum, (B) whole blood, and (FDB) freeze-dried whole blood.
[b]Data exclude Pb + Se treatment values.
[c]Data exclude Pb treatment values.

DISCUSSION

The depressive effects of Pb on feed intake and body weight measured in this study have been observed in many species (*1*). However, in another study, Pb (as lead acetate) at concentrations of up to 1000 μg/g in a practical diet fed to young lambs for 84 d did not affect feed consumption, weight gain, or the feed conversion ratio (*12*). Their diet, assuming 1.3 kg dry matter intake by 36 kg lambs, provided a Pb intake of 36 mg/kg b.w., which is 3.6 times the level administered to the adult sheep in this study. Young animals are more sensitive to Pb toxicosis than are mature adults (*1*), but possibly these 36 kg lambs should not be considered as young animals. Dietary Ca concentrations were also higher in our study than for the lambs (2.0% vs estimated 0.14% Ca), which should have reversed the Pb effects on those two classes of sheep. Practical diets were used in both studies, although the lambs were fed a high concentrate ration and they were offered 10% more feed than was consumed the previous day. The Pb was mixed in the feed for the lambs, whereas it was provided in an encapsulated dose in this study. The dosing treatment may

have aggravated the toxicity effects of Pb. Differences in the Pb source may also have been a factor.

The weight loss by these adult sheep may have been a direct effect of reduced dietary intake, an indirect effect of Pb on animal metabolism, or both.

Selenium, as the selenite has been shown to protect against Pb toxicosis in some cases (*2,3*). Yet, in other cases Se had no effect (*5*) or increased Pb toxicosis (*2*), which was also demonstrated in this study. The confusion associated with this interaction of Se with Pb is not readily explained by intake rates of either Pb or Se, nor by the ratio of the two (Table 2). Perhaps in our study the daily intake of 3.6 mg Se/animal/d (2.2 μg Se/g diet for animals eating the 1.6 kg allowance) was excessive, and an intake of 1 mg Se/animal might have been more appropriate. Se was mildly protective (*2*) against the toxic effects of Pb only up to 0.50 μg

TABLE 2
The Role of Se on Pb Toxicosis for Given Levels of Pb and Se Intake (μg/kg b.w./d) as Calculated from Publishing Data

Source	Pb Intake	Se Intake	Pb:Se	Role of Se in Pb toxicosis
	Rastogi et al.[a] (1976)			
Rats	100,000	370	270	Protective
	100,000	650	150	Protective
	Cerklewski and Forbes[b] (1976)			
Rats	12,800	.96	13,000	Mildly protective
	12,800	3.2	4000	Mildly protective
	12,800	32	400	Mildly protective
	12,000	64	200	Additive
	Stone and Soares[c] (1976)			
Japanese quall	500	1	5000	No effect
	1000	1	1000	No effect
	Mayland et al. (this manuscript)			
Sheep	9,800	58[d]	170	Additive

[a]During weeks 4–8, Pb was administered by cutaneous application as 200,000 μg[redacted] Pb/kg-animal every other day. Selenium was given at 5 and 10 μ/g in drinking water.

[b]During week 6, Se concentrations were 0.015, 0.05, 0.5, and 1.0 μg Se/g diet whereas Pb was 200 μg/g diet. These values correspond to approximately 0.96, 3.2, 32 and 64 μg Se and 12,800 μg Pb/kg b.w.-d, respectively.

[c]Concentration in diet, μg/g.

[d]The value is calculated as 0.64 mg Se from 1.6 kg alfalfa plus 3 mg supplementary Se per 63 kg sheep.

Se/g. At higher Se concentrations, an exaggeration of Pb toxicosis was observed. Although the source of supplemental Pb and Se will be a factor in determining availability, the ratio of absorbed Pb to absorbed Se may be more important and meaningful than the Pb/Se intake. It is well established that mineral absorption varies between animals species and between young and adult animals (*1*). Because delta-aminolevulinic acid derydratase (ALAD) activity in tissue is a useful parameter in the study of Pb toxicosis and Pb–Se interactions, it is unfortunate that its activity was not measured in blood and soft tissue in this study.

The additions of both Se and Pb to the diet resulted in higher concentrations of Pb and Se in some tissue than when either element was fed alone (refs. *3* and *4*, and this study). However, Se levels in some soft tissue were decreased by the interactive effects of Pb and Se (refs. *2* and *4*, and this study). The results of dietary Pb and Se on tissue levels of these two elements must be related to the absolute levels of soluble Pb, Se, and other interactive elements, and the proteins and or chelating agents in the diet or in the gastrointestinal tract.

The data in this study clearly illustrate that Se increased the toxicity of Pb when fed to sheep at the rate of 3 mg Se/animal/d and 9.8 mg Pb/kg b.w./d, respectively. Hence, a protective effect of Se against Pb toxicity is not confirmed by this research.

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