Evaluation of orally administered calcium carbonate and zinc sulphate on the gastrointestinal absorption of lead acetate in cattle.

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Abstract: Lead is one of the most frequently observed heavy metal toxicoses in domestic animals, especially cattle. The objective of the study was to test the effect of calcium carbonate and zinc sulphate on the absorption of lead acetate in cows, when the lead and minerals were both administered orally. In study 1, experimental cows were dosed with lead acetate at 2 mg/kg plus calcium carbonate at 50 mg/kg, while control cows received only lead acetate at 2 mg/kg. During study 2, experimental steers received zinc sulphate at .2 mg/kg and lead acetate at 2 mg/kg, while the control steers received lead acetate at 2 mg/kg. Blood samples were taken in heparin tubes from the jugular vein at 0, 3 and 6 hours post-dosing.. Blood lead concentrations were then determined by atomic absorption spectrophotometry, using the graphite furnace method. The results indicated that both minerals decreased the absorption of lead acetate in cows after 3 hours and the effect was sustained at 6 hours. The recommendation to farmers is to provide supplementary minerals to animals in areas with high risk of lead poisoning.
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Introduction

Lead is one of the major environmental pollutants, causing unintentional poisoning more frequently than any other substance (Priester and Hayes 1974). Sources of lead are batteries, boiled linseed oil, paint, putty, grease, lead arsenate pesticides and foliage near lead smelters (Gwaltney-Brant 2004; Smith 1990). It is projected that 98% of airborne lead in The United Kingdom originates from leaded petrol (Timbrell 1997). In all countries that have banned leaded gasoline, average blood lead levels have dropped sharply (Meyer et al. 2008). However, some developing countries still allow leaded gasoline (Payne 2008), which is the primary source of lead exposure in most of the developing countries (Meyer et al. 2003). About one fifth of the world's disease burden from lead poisoning occurs in the Western Pacific and another fifth in Southeast Asia (Payne 2008).

Numerous reports of lead poisoning have been reported worldwide. Examples include feeder calves exposed to lead (Osweiler and Ruhr 1978) and cattle eating forage contaminated by sandblasted paint (Every and Nicholson 1981). Records from eleven veterinary colleges in the United States and Canada indicated 162 cases over a four year period (Priester and Hayes 1974). In 2006, the Onderstepoort Veterinary Institute (OVI) diagnosed seven positive cases in cattle and four positive cases in cage birds (OVI unpublished data). This is the tip of the iceberg, since many cases are not reported. The OVI records for more recent years will probably also not reflect the true incidence of cases.

Lead poisoning occurs relatively regularly in humans, mammals, birds, and reptiles, while swine, goats and chickens are more resistant to the effects of lead. Ruminants tend to show central nervous dysfunction, whereas equines usually display signs of peripheral neuropathy. Dogs and cats show both neurological and gastrointestinal signs (Gwaltney-Brant 2004).

Lead absorption can be enhanced in calcium, zinc, iron and vitamin D deficient animals (Gwaltney-Brant 2004). Elevated dietary calcium, phosphorus, iron, zinc, fat and protein decrease the absorption and retention of lead (National Research Council 2001). Suboptimal calcium or phosphorus in horses can increase absorption and retention of lead (Seawright *et al*. 1983), while zinc, copper and magnesium block lead uptake (Ballantyne *et al*. 1993).

However, care should be taken to supply the correct amounts of some nutrients, because high levels of iron tend to increase the absorption of lead (Garrettson 1983), while high levels of vitamin D also increase lead absorption (Smith 1990). Oral administration of 25 i.u. of cholecalciferol to weanling rats increased absorption of lead acetate by 33 percent (National Research Council 1980). The objective of the trials was to establish if orally dosed

copper sulphate can decrease the absorption of lead from the gastrointestinal tract.

Materials and methods

Experimental animals

Cows were kept in an enclosure with short grass cover and received a hay mixture of Cenchrus cilliaris (blue buffalo grass) plus luserne. A concrete basin contained a flotation device to refill with fresh water. The broilers were fed commercial broiler finisher pellets and water ad lib.

Ethical considerations

Lead poisoning in cattle has been induced in cattle by feeding 6 mg /kg of lead acetate for 7 days (Smith 1990). The dose of lead acetate in the trials was 2 mg/kg in all the trials once-off only, therefore no animal was unduly harmed.

Studies

Base-line blood lead measurements were done on all animals in all the trials to establish if they could have been exposed to lead. The majority of the pre-dosing samples measured zero lead, and the highest value was 0.004 ppm. The zero hour values were deducted from the post-dosing values.

Study 1

Eight Friesland cows in the treatment group received lead acetate at 2 mg/kg and calcium carbonate into the rumen with a 16G catheter at 50mg/kg, while the control group only received lead acetate at 2mg/kg. The treatment group and the control group were bled from the jugular vein using heparin tubes. The blood lead levels were determined at 0, 3 and 6 hours post-dosing.

Study 2

Eight Holstein steers in the treatment group received zinc sulphate at 2 mg/kg plus lead acetate at 2 mg/kg, both intraruminally while the controls only received lead acetate at 2 mg/kg into the rumen. Blood samples were taken at 0, 3 and 6 hours.

Atomic absorption spectrophotometer

The blood samples were analyzed by atomic absorption spectrophotometry and the model used in the trials was the AAnalyst 700. The wavelength was set at 283.3 and the slit width at 0.7L (low) for the furnace method. The lead method was created by using Windows 2000 Winlab 32 program.

Graphite furnace method (Khullar 2002)

Sample preparation

A diluent of "TritonX-100" 0.1% (w/v), ammomium dihydrogen phosphate 0.2% and ammonia 0.14M was prepared in deionized water. Blood samples were mixed with the diluent 1: 10 in 1ml polystyrene autosampler cups.

Standards

A working standard solution 10ppm was prepared in 0.2% nitric acid. The standard curve was obtained by diluting the working standard in 0.2% nitric acid and 0.2% ammomium dihydrogen phosphate solution to obtain 0.125 ppm, 0.25 ppm and 0.5 ppm standards. The calibration blank was 0.2% nitric acid and 0.2% ammomium dihydrogen phosphate solution and the reagent blank was the diluent solution.

Furnace conditions

Step#	Temperature. (^{O}C)	Ramp Time (sec)	Hold Time (sec)	Internal Flow (ml/min)	Gas Type
1	120	10	20	250	Argon
2	130	5	30	250	"
3	700	15	30	250	"
4	20	1	15	250	"
5	1700	0	5	0	"
6	2600	1	5	250	"

Statistical analysis

The one-tailed t-test was done to test the hypothesis that the blood lead levels of the experimental calves are lower than the control calves ($\mu_1 < \mu_2$). The means and standard deviations of the experimental values and the control values were calculated. Thereafter, further calculations were done such as the difference between the means and the standard error of the difference between the sample means. Finally the test statistic for 12 degrees of freedom provided a numerical number, which gave an area under the curve (Samuals and Witmer 1999). The level of significance was chosen to be $P < 0.05$.

Results

The graphs show solid lines for the sake of visual clarity, but the values were not continuous data. The dots are the actual values as individual point measurements. This applies to all the following graphs as well. Three hours post-dosing, all the treatment cows had lower blood lead concentrations than the control cows and the differences were highly significant ($P < 0.05$). The differences between the two groups were approximately three-fold in most instances, therefore calcium showed major competition for absorption sites in the intestinal mucosa during the first three hours after dosing (Figure 1).

The gap between the two groups was smaller than the 3 hour time slot, but still highly significant at 6 hours ($P < 0.05$). Even when the effect of calcium was reduced, it showed that the treatment group had not yet caught up with the control group regarding blood lead levels at that point in time. Most of the treatment group showed a two-fold or more increase in blood lead levels between 3 and 6 hours post-dosing, whereas the control group showed 30% or less increase during the same period. The indication is that calcium delays the absorption of lead, but this effect weakens over time and that unless faecal excretion may occur within 6 hours of lead ingestion, the lead still in the intestine after 6 hours will eventually be absorbed as well (Figure 2).

All the experimental steers had lower blood lead concentrations than the controls. The control group values fluctuated more than the experimental group, indicating that zinc had a reasonably constant effect on lead absorption in the treatment group. The differences were highly significant ($P <0.05$). zinc did not display the same dramatic effect as calcium in the first 3 hours after dosing, but the usefulness of zinc at this point is commendable (Figure 3).

The graph of 6 hours appears to be very similar than the 3 hour graph. The respective blood lead levels were just higher for the 6 hour measurements for both groups and the differences between treatment and control groups were highly significant ($P< 0.05$). The treatment group absorbed very little additional lead between the time frame 3 and 6 hours post-dosing. In the control group, most steers absorbed relatively little additional lead, except for two individuals absorbing more than the rest during the same period of between 3 and 6 hours (Figure 4).

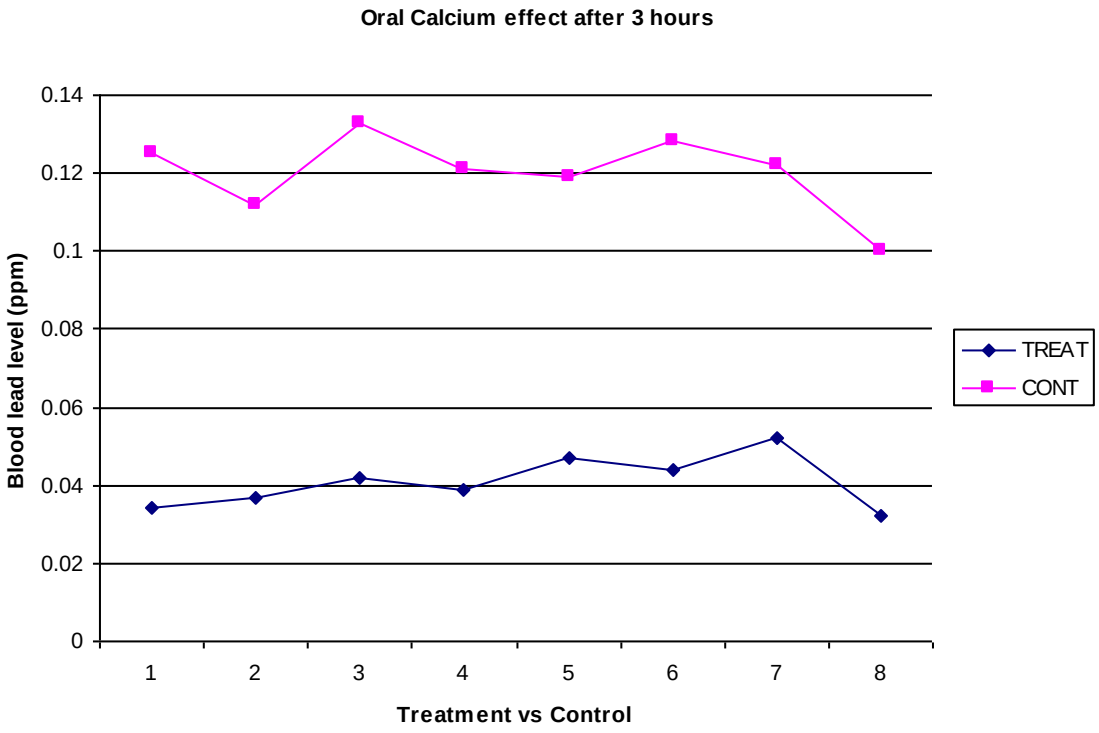

Figure 1. Lead administered at 2mg/kg to all cows and calcium carbonate given to the treatment group at 50mg/kg

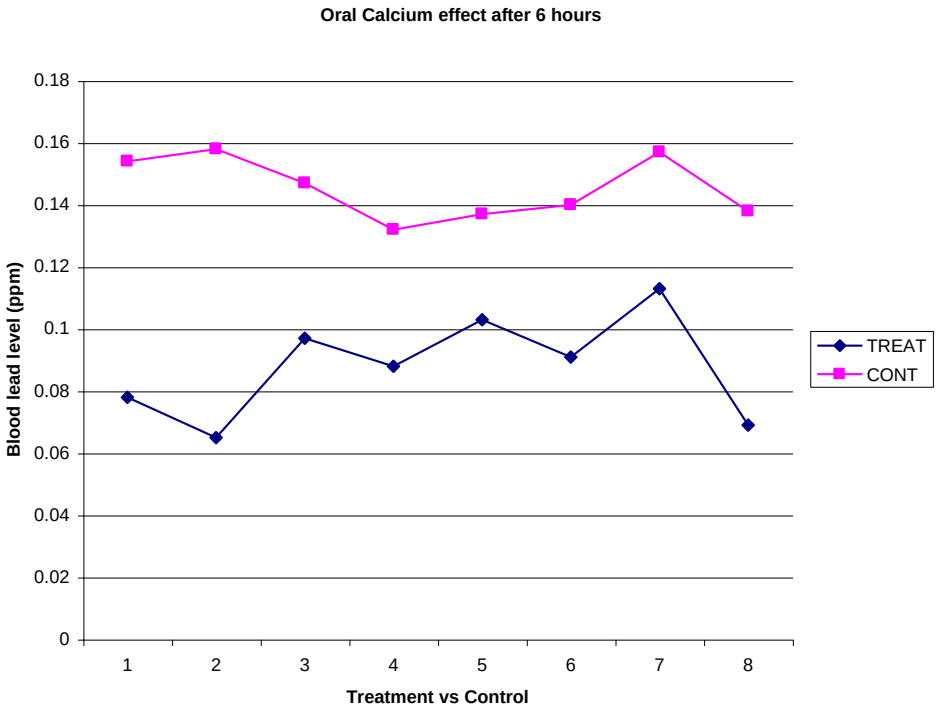

Figure 2. Lead administered at 2mg/kg to all cows and calcium carbonate given to the treatment group at 50mg/kg

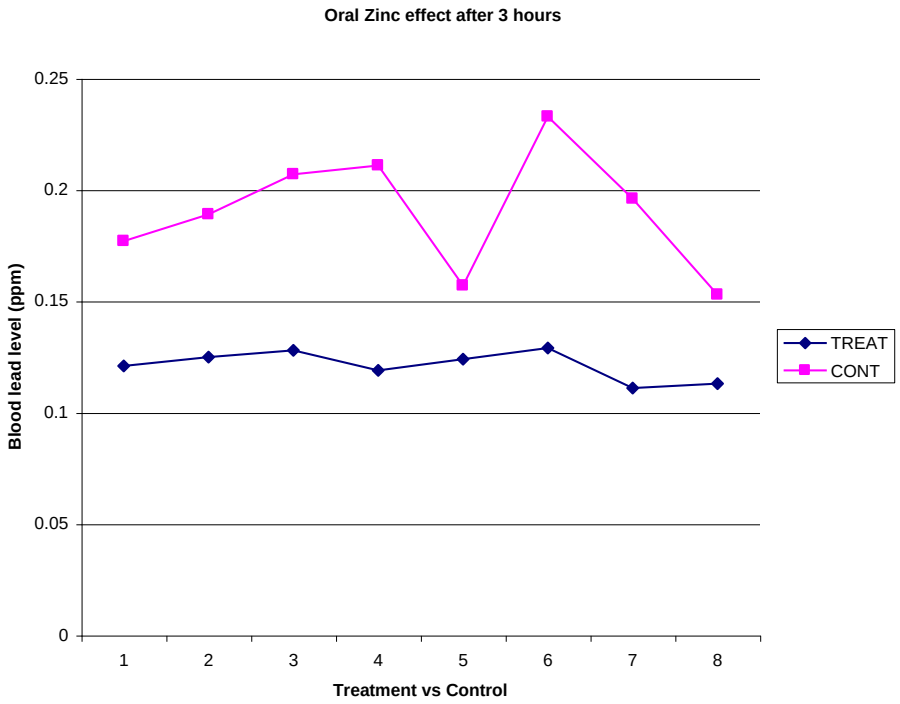

Figure 3. Lead administered at 2mg/kg to all steers and zinc sulphate given to the treatment group at 2 mg/kg

Figure 4. Lead administered at 2mg/kg to all steers and zinc sulphate given to the treatment group at 2 mg/kg

Discussion

Lead ions antagonize the action of Ca^{2+} ions at many sites and mimic many of the biological effects calcium ions, but lead has many effects different than those of calcium, so that the toxicity of lead cannot be explained solely by its interaction with calcium (Simons 1986). When minerals are added to the feed, many factors can influence the effectiveness of such a program. Not only the amounts of minerals required, but also their availability, interaction with each other, ratios, tolerances, toxicities and sources must be considered ((Russof 1981).

Several trials indicated the interactions between calcium, phosphorus and lead. Low dietary intake of phosphorus in chickens increased both calcium and lead absorption as well as increased the synthesis of calcium-binding proteins, when chickens were treated with cholecaciferol. Bovine and chick vitamin D_3 induced intestinal calcium-binding proteins which can bind lead. Calmodulin, troponin C and oncomodulin bind lead with high affinities, even in preference to calcium (Fullmer *et al*. 1985). In a trial with young, growing chickens, dietary lead intake resulted in two independent and opposite effects on intestinal calcium and lead absorption, depending on dietary calcium and lead levels and duration of treatment. In calcium deficiency, calcium supplementation resulted in more calcium being absorbed, regardless of lead intake (response after 1 week) but later in the trial (2 weeks) lead inhibited the same degree of calcium absorption. Calcium deficiency without calcium supplementation resulted in more lead being absorbed (Fullmer 1991).

A trial in fish (Cirrhinia mrigala) showed that calcium decreased lead absorption and reduced the accumulation of lead in muscles (Lopa and Adhikari 2006). The mechanism of branchial lead uptake and interplay with calcium transport was also investigated in the freshwater rainbow trout (Oncorhynchus mykiss). It was observed that branchial lead accumulation was reduced with increased waterborne calcium concentrations. Cadmium and zinc were used as voltage-independent calcium channel competitors and this also reduced brachial lead uptake. This suggests that lead is transported through these channels and when lead levels become high, it disrupts calcium influx and

ultimately calcium homeostasis (Rogers and Wood 2004).

Although adequate calcium is recognized as a preventative measure in lead-exposed populations, little is known about the effect of calcium supplementation above normal dietary requirements on lead metabolism, especially in young, suckling animals. Gastrointestinal absorption of cations, including lead and calcium is much higher during the suckling period. In a trial of suckling rats, calcium supplementation caused statistically significant reduction in lead concentrations of liver, kidneys, brain and carcass (Varnai *et al*. 2001). In another trial in adult rats, the uptake of lead was reduced by about half when either dietary calcium or phosphate or both was doubled (Quarterman et al. 1978).

An inverse relationship between exists between brain lead and dietary calcium, confirming that calcium deficiency not only elevates blood lead levels, but increases lead in the most sensitive organ in infants and young children. Excess calcium also lowered blood lead levels, but in a less dramatic way than the enhancement of lead toxicity in calcium deficient diets (Goyer 1978).

Lead blocks entry of calcium into nerve terminals and impairs normal calcium homeostasis in cells, by competing with calcium for the uptake by calcium channels. Lead also blocks calcium efflux from cells by substituting for calcium in calcium-sodium ATP pumps. The other mechanism is lead competing with calcium for calcium-binding proteins (Goyer 1997)

The beneficial effect of high zinc on lead toxicity has been observed in horses (Schmitt *et al*. 1971, Willoughby *et al*. 1972) and in rats (Cerklewski and Forbes 1976a), but in swine high levels of zinc enhanced the toxicity of lead (Hsu *et al*. 1975). There is a little information concerning the interrelationship of zinc and lead in cattle. A study on calves fed 1500 ppm lead acetate produced decreased zinc levels in all tissues, except tibia, muscle and brain (White et al. 1985).

Aminolevulenic acid dehydratase (ALAD) is an allostering enzyme consisting of eight subunits, each containing one zinc atom and eight sulfhydryl groups. Lead is believed to inactivate ALAD by replacing zinc in a stoichiometric manner. On the other hand, the inhibition of ALAD by lead can be reversed by addition of zinc and reducing agents such as gluthione and dithiotheitol. Glutathione removes lead from sulfur groups on the enzymes and may reduce oxidized sulfur groups; while supplementation with zinc may displace lead from the enzyme active site (Goering et al. 1987). Oral administration of zinc sulphate following chelation therapy has been found to significantly increase δ-ALAD activity (Goyer 1997).

It is not known how very high levels of certain minerals may influence lead absorption, but at least some leeway is possible with respective dosages. The recommendation to farmers would be to feed adequate levels of minerals in high risk areas for lead poisoning. However, minerals have a limited capacity to delay lead absorption, especially after 6 hours when most of the mineral itself is already absorbed. Further advice would be to prevent cattle eating lead containing objects by fencing off rubbish dumps and not to use lead-based paints on gates and buildings, causing paint flakes to be ingested by cattle.

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