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# Lactose and Milk Replacer Influence on Lead Absorption and Lead Toxicity in Calves

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The absorption, tissue deposition, retention, and excretion of ingested lead is in large part due to associated dietary factors. Young suckling calves are extremely susceptible to low doses of lead, especially when maintained totally on milk (Bratton et al. 1981; Zmudzki et al. 1983, 1984). Unfortunately, the complexity of milk makes it difficult to determine which constituent is actually responsible for increased Pb absorption.

Milk itself has been reported to increase intestinal Pb absorption in young and mature rats (Kello and Kostial 1973; Kostial et al. 1978). In contrast, others have reported that milk has no effect on Pb absorption in mice (Garber and Wei 1974), and retards rather than increases the intestinal uptake of Pb in rats (Henning and Leeper 1984). Recent studies have shown that lactose, the major carbohydrate of milk, is a dietary factor that increases the absorption of several minerals including Pb (Bell and Spickett 1981; Bushnell and DeLuca 1981, 1983) in rats. Our laboratory has recently demonstrated that milk greatly increased the tissue deposition of lead in calves (Zmudzki et al. 1984). Since all dietary vitamins and minerals were adjusted to recommended levels in all treatment groups, the influence of minerals and vitamins was eliminated from concern. Lactose, however, has not been considered in the ruminant animal. Moreover, liquid milk seems to increase the absorption of lead more significantly than powdered milk (Kello and Kostial 1973). The purpose of this study was to assess the influence of lactose and powdered milk on lead uptake and tissue distribution in calves.

## MATERIALS AND METHODS

Fifteen 1-2 week old Holstein-Friesian bull calves weighing approximately 50 kg were used in this study. The calves were individually housed in concrete-floored wire pens and initially fed three times daily with a commercial milk replacer diet. Following a 7-day acclimatization period, 12 calves were gradually

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transferred, over a 10-day period, from the milk replacer diet to a diet consisting of grain calf starter and shredded coastal Bermuda grass hay. The hay was added to the ration at 10% by weight. Three calves were continued unaltered on the liquid milk replacer diet. Dietary factors in the milk replacer and grain-hay diets used in this experiment were previously published (Zmudzki et al. 1984). Lead levels in these diets were below 0.1 mg Pb/kg dry diet.

After the calves on grain-hay diets had adjusted (14 days), the calves were divided into 5 different dietary groups consisting of 3 calves per group. Group I calves were fed a grain-hay diet, Group II calves were fed a grain-hay diet containing 10% powdered lactose; Group III calves were fed a grain-hay diet containing 40% powdered lactose; Group IV calves were fed a grain-hay diet containing 40% powdered milk replacer; and Group V calves were continued on a total liquid milk replacer diet via nursing bottle. All calves were dosed with 5 mg Pb/kg b.w./day using lead acetate ($Pb[C_2H_3O_2]_2.3H_2O$ - Fisher Scientific Company, Houston, TX) for 7 days. The Pb was dissolved in 100 ml of water and given via nursing bottle approximately 2 hours after the morning feeding. Data regarding non-Pb-dosed controls have previously been published (Zmudzki et al. 1983).

Heparinized blood samples (20 ml) were drawn from the external jugular vein of all calves 3 days prior to dosing with Pb in order to establish baseline values for BPb, erythrocytes δ-aminolevulinic acid dehydratase (ALAD) activity, hemoglobin, packed cell volume, total erythrocytes, total leukocytes and serum iron, zinc, copper, magnesium, calcium, and phosphorus. Blood samples were collected 1, 3, 6, 12, and 24 hours after the first dose of lead and then 24 hours following the second, fourth, and seventh doses of Pb. Physical examinations were performed and recorded at all daily feedings.

All surviving calves were killed via an IV injection of pentobarbitol (Nembutal Sodium, Abbott Laboratories, North Chicago, IL) on the eighth day of the experiment. The calves were exsanguinated and kidneys, liver (left lobe), spleen, pancreas, biceps femoris muscle, heart (ventricle), cerebellum, cerebrum, sciatic nerve, spinal cord (thoracic portion), lung (left caudal lobe), bone (left femoral head and left 4th rib), urine (50 ml), and bile were collected for Pb analysis. Similar samples were collected within 8-12 hours after death from all calves that died during the study.

Analysis of dietary Pb, blood Pb, tissue Pb, hematological parameters, and serum minerals followed methods previously described (Bratton et al. 1981). The data were analyzed by a paired t-test and by two-way analysis of variance with Duncan's multiple range test used to determine significance of differences between means of the different groups of animals.

## RESULTS AND DISCUSSION

After 7 doses of Pb, all calves on the liquid milk diet showed signs of Pb intoxication and one died on the seventh day of study.

No calves from the other groups showed any neurological or gastrointestinal signs of Pb poisoning.

Blood lead data are shown in Table 1. The peak BPb, following the initial Pb exposure, occurred in all calves regardless of diet at approximately 6 hours. The peak blood Pb was significantly elevated ($P<0.001$) at 6 hours in the calves fed liquid milk compared to all other animals. By 7 days, blood Pb was significantly higher ($P<0.05$) in all groups receiving milk or lactose compared to the calves fed only grain and hay. The greatly elevated blood Pb in the liquid milk-fed calves indicates a greater absorption of Pb in these calves and explains why clinical signs were observed only in these animals.

Table 1. Blood lead concentration in calves treated with 5 mg Pb/kg b.w./day for 7 days expressed as µg/dl, x SD (n = 3 in each group)

| Time | Grain-hay only | Grain-hay + 10% lactose | Grain-hay + 40% lactose | Grain-hay + 40% milk repl. | Liquid milk repl. only |
|---|---|---|---|---|---|
| Pretreatment | 2± 1 | 2±0 | 2±0 | 2± 1 | 2± 1 |
| 1 hour | 8± 4 | 3±0 | 4±0 | 12± 8 | 7± 4 |
| 3 hours | 22±22 | 8±3 | 16±7 | 15±17 | 34±22 |
| 6 hours | 40±21 | 15±4 | 21±9 | 26±10 | 111±60 |
| 12 hours | 30±12 | 18±6 | 22±9 | 23± 4 | 65±17 |
| 24 hours | 21±10 | 15±2 | 17±3 | 21± 5 | 48± 7 |
| 2 days | 27± 8 | 24±3 | 27±2 | 30± 5 | 67± 2 |
| 4 days | 26± 5 | 34±2 | 39±5 | 43± 5 | 86± 6 |
| 7 days | 32± 7 | 44±4 | 45±5 | 52±12 | 104±25 |

It is interesting to note that in all groups receiving grain and lactose or powdered milk, there was a uniform daily increase in BPb of approximately 5 µg/dl. This increment was doubled (10 µg/dl) in calves receiving liquid milk, while the calves fed grain and hay seemed to hold at approximately 1 µg increase/day.

After 7 days of exposure to Pb, BPb was significantly increased ($P < 0.01$) in every calf when compared to pre-exposure levels. However, BPb was significantly less ($P < 0.05$) elevated in calves receiving only grain-hay than in calves receiving lactose or milk replacer with or without grain-hay. This suggests that milk and lactose both increase the absorption and blood concentrations of Pb. Since BP was almost identical in calves receiving 10% and 40% lactose or 40% milk replacer, we concluded that lactose does increase Pb absorption in ruminants and is the major factor in milk responsible for increased Pb absorption. The marked elevation in BPb in calves receiving liquid milk also indicates that liquid milk increases Pb absorption to a highly significant degree as shown previously in the preruminant calf. It is critical now to further investigate the effect liquid milk would have in calves

receiving grain and hay and in cattle with fully developed forestomachs.

These data in calves are similar to the findings of Kello and Kostial (1973), Bushnell and DeLuca (1981, 1983), and Bell and Spickett (1981) who indicated that milk and lactose increase Pb absorption and tissue deposition in rats. They are different from the studies of Garber and Wei (1974) and Henning and Leeper (1984) who indicated that milk either did not affect Pb absorption or actually decreased Pb absorption. In these latter two papers, no consideration was given to the solid components of the diet which may in fact have overshadowed the effects of lactose or milk.

Lead effects on ALAD activity in erythrocytes are shown in Table 2. By 6 hours, ALAD activity had dropped to approximately 20% of pretreatment levels in all calves regardless of diet. A further decrease to approximately 10% of pretreatment levels had occurred by 7 days of lead exposure. There was no dietary influence on Pb in regard to its effect on ALAD. This illustrates the high sensitivity of ALAD to Pb and the inability of ALAD inhibition to measure the exposure levels of Pb in cattle.

ALAD inhibition levels were almost exactly the same in all animals after 7 days of Pb exposure, but blood Pb and tissue levels were remarkably different. We conclude that ALAD is remarkably sensitive to Pb and the effect on inhibition was maximal even in calves receiving grain and hay that had only a 32 µg/dl BPb level. ALAD does not indicate the current BPb level in the calf as has been suggested for the rat (Bell and Spickett 1981). ALAD is also not a good indicator of body Pb burden, but it does show a distinct metabolic influence of Pb.

Together with blood Pb, ALAD has great diagnostic potential in veterinary diagnostic laboratories charged with laboratory confirmation of Pb poisoning in cattle. For example, 32 µg/dl of Pb in the blood of calves on grain and hay is in the questionable range for clear diagnosis of lead poisoning when BPb is the sole parameter evaluated from calves showing clinical signs of poisoning. Simultaneous measurement of ALAD activity would clearly resolve this questionable blood level into a positive diagnosis of Pb poisoning because of the profound effect Pb has on ALAD activity.

All calves from the groups which had grain and hay in the diet accumulated significantly ($P<0.01$) less Pb in tissues than did the calves on liquid milk replacer (Table 3). However, calves fed 40% lactose or 40% milk replacer accumulated 2 times more Pb in the tissues than calves on grain-hay or grain-hay plus 10% lactose. For all tissues, there was a trend for increased tissue accumulations of Pb as the diet contained increasing levels of milk or lactose. The almost equal levels of tissue Pb accumulation between calves receiving grain and hay plus 40% lactose or 40% milk replacer would again suggest that lactose is the main component of milk that increases the tissue accumulation of Pb in calves.

Our research, like that of Kello and Kostial (1973), indicates that milk causes a striking increase in Pb tissue distribution

Table 2. Erythrocyte ALAD activity in calves dosed with 5 mg Pb/kg b.w./day for 7 days expressed as a percentage of pretrial values, $\bar{x}\pm SD$ (n = 3 in each group)

| Time | Grain-hay only | Grain-hay + 10% lactose | Grain-hay + 40% lactose | Grain-hay + 40% milk repl. | Liquid milk repl. only |
|---|---|---|---|---|---|
| Pretreatment | *527.6±145.0 | 670.0±160.0 | 359.0±100.1 | 436.1±282.8 | 494.9±95.1 |
| Value | 100% | 100% | 100% | 100% | 100% |
| 1 hour | 76.8±5.3 | 87.8± 3.5 | 82.9±11.7 | 70.9±21.6 | 90.7±34.3 |
| 3 hours | 28.6±3.8 | 51.2±13.1 | 37.2±15.8 | 33.5±19.9 | 42.7±34.0 |
| 6 hours | 15.4±3.6 | 31.1± 9.3 | 20.9± 8.8 | 19.6±11.6 | 11.3± 9.7 |
| 12 hours | 12.0±2.3 | 21.3± 5.5 | 16.4± 2.3 | 13.0± 2.4 | 12.7±12.2 |
| 24 hours | 21.3±7.2 | 22.8±10.0 | 23.6± 2.0 | 17.3± 8.7 | 15.5± 1.7 |
| 2 days | 12.3±0.8 | 14.0± 1.4 | 11.3± 2.1 | 13.7± 7.1 | 16.4± 4.4 |
| 4 days | 14.6±2.3 | 15.1± 2.8 | 13.2± 2.3 | 14.9± 8.2 | 14.3± 3.4 |
| 7 days | 16.4±2.9 | 11.9± 1.3 | 11.9± 1.3 | 13.4± 5.0 | 14.9± 2.4 |

*nM porphobilinogen/ml red blood cells/hr

Table 3. Lead concentration in calf tissues following 7 days of lead exposure expressed as mg/kg, $\bar{x} \pm SD$ (n = 3 in each group)

| Tissue | Grain only | Grain + 10% lactose | Grain + 40% lactose | Grain + 40% milk repl. | Milk repl. only |
|---|---|---|---|---|---|
| Kidney (total) | 9.89±7.20 | 10.20± 5.61 | 24.58 ±13.24 | 19.45 ±11.78 | $104.28^{c}$±33.81 |
| Liver | 20.02±9.77 | 19.93±13.69 | 31.13± 8.87 | 38.92±28.98 | $36.42^{b}$±15.56 |
| Spleen | 0.49±0.08 | 0.39± 0.06 | $1.15^{b}$± 0.24 | $0.93^{b}$± 0.30 | $1.19^{b}$± 0.13 |
| Pancreas | 0.89±0.20 | 1.15± 0.31 | $3.51^{b}$± 1.74 | $1.93^{a}$± 0.44 | $6.26^{c}$± 2.72 |
| Muscle | 0.08±0.03 | 0.07 ± 0.01 | 0.15± 0.01 | 0.14 ± 0.01 | $0.26^{c}$± 0.08 |
| Heart | 0.11±0.03 | 0.11 ± 0.02 | $0.21^{b}$± 0.04 | 0.20 ± 0.03 | $0.51^{c}$± 0.18 |
| Lung | 0.36±0.04 | 0.31 ± 0.11 | $0.67^{a}$± 0.01 | $0.58^{a}$± 0.11 | $1.43^{c}$± 0.13 |
| Testes | 0.15±0.02 | 0.19 ± 0.05 | $0.31^{b}$± 0.11 | $0.30^{b}$± 0.06 | $1.13^{c}$± 0.25 |
| Sciatic N. | 0.19±0.05 | 0.16 ± 0.02 | $0.33^{b}$± 0.05 | $0.31^{b}$± 0.02 | $0.99^{c}$± 0.22 |
| Cerebellum | 0.18±0.01 | 0.24 ± 0.08 | $0.39^{a}$± 0.13 | $0.35^{a}$± 0.09 | $0.60^{c}$± 0.15 |
| Brain Stem | 0.13±0.02 | 0.15 ± 0.02 | $0.27^{c}$± 0.03 | $0.27^{c}$± 0.02 | $0.72^{c}$± 0.29 |
| Cerebrum | 0.18±0.01 | 0.18 ± 0.05 | $0.39^{c}$± 0.08 | $0.37^{c}$± 0.04 | $0.71^{c}$± 0.17 |
| Spinal Cord | 0.08±0.02 | 0.08 ± 0.01 | $0.15^{b}$± 0.03 | $0.18^{b}$± 0.02 | $0.30^{c}$± 0.06 |
| Rib | 4.29±0.74 | 5.74 ± 1.53 | $12.55^{b}$± 4.69 | $9.44^{a}$± 2.15 | $57.26^{c}$±12.32 |
| Femoral H. | 5.81±1.49 | 6.71 ± 1.41 | $13.21^{b}$± 3.01 | $13.11^{b}$± 2.27 | $71.96^{c}$±26.77 |
| Urine | 0.06±0.02 | 0.10 ± 0.06 | 0.11 ± 0.08 | 0.11 ± 0.04 | 0.43 ± 0.38 |
| Bile | 0.37±0.08 | 0.40 ± 0.26 | 0.58 ± 0.14 | 1.66 ± 1.17 | $3.25^{a}$± 1.94 |
| Blood | 0.32±0.07 | $0.44^{a}$±0.04 | $0.45^{a}$± 0.05 | $0.52^{a}$± 0.12 | $1.04^{c}$± 0.25 |

a = $P < 0.05$, b = $P < 0.01$, c = $P < 0.001$, compared to grain-hay group.

presumably due to greatly increased absorption. We also observed that liquid milk seems to increase the absorption of Pb much more than powdered milk as has been described in the rat (Kello and Kostial 1973), but we did not have a group of calves fed grain-hay with liquid milk.

Milk in rats fed soy meal increased Pb in kidney, liver, and brain (Bell, Spickett 1981). We also found a marked increase in lead in these tissues in calves receiving only milk, but only the brain levels of Pb were significantly elevated in calves receiving grain-hay and milk or lactose. The liver and kidney levels of lead were not significantly affected.

Physiological levels of lactose in rats (80 mM) exerted a protective effect against the absorption of ingested Pb (Bushnell and DeLuca, 1983). Lactose in the levels present in cow's milk did not protect calves from absorption of Pb and in fact elevated the absorption significantly over a diet of grain-hay (Zmudzki et al. 1985). Tissue levels in these calves were also markedly elevated, but retention levels over time were not evaluated.

It is interesting to note that as the levels of lactose or milk increased in the diet, the excretion levels of Pb in the bile increased; yet the liver Pb levels were not different. The variability was high, but the trends were definitely present. On the other hand, milk and lactose did not affect urine Pb levels.

The relationship of lead in the kidney and liver was greatly changed in the calves receiving only milk. The liver lead levels were all elevated approximately 2 times above kidney Pb levels in calves receiving grain and hay, while in calves receiving liquid milk only, the Pb in kidney was 3 times greater than the liver Pb concentration.

The Pb levels in bone increased with increasing levels of Pb in the blood and with increasing levels of lactose or milk in the diet. This is in contrast to studies in the rat where lactose reduced the levels of Pb in bone (Bushnell and DeLuca 1981).

While this study was not designed to evaluate the rate of Pb absorption, the marked elevation in blood Pb in calves receiving lactose or milk would indicate that milk and lactose do increase the absorption of Pb.

Fasting was not a part of the study. Diarrhea and changes in body weight were not seen as reported in the rat given lactose (Bushnell and DeLuca 1983), and these factors can be eliminated. Whether the narrow window effect of lactose on Pb absorption seen in rats (Bushnell and DeLuca, 1983) occurs in cattle needs to be defined.

Lengemann et al. (1959) indicated that in order for lactose to increase calcium absorption it had to be present in the same intestinal segment at the same time. Bell and Spickett (1981) showed that in order for lactose to increase levels of Pb in

tissue, milk and Pb had to be consumed at the same time. This study definitely shows that in calves, Pb and milk do not have to be fed at the same time, as the Pb was given 2 hours following the 1st feeding period. However, it may well be that in the calf these two substances were in the same gut segment coincidentally even though the substances were not ingested together.

While these studies show that milk and lactose affect the absorption and tissue distribution of Pb, there are still a number of unanswered questions. Diet, however, plays a critical role in lead absorption states and must be very carefully evaluated in cases of Pb poisoning in both man and animals.

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