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THE EFFECT OF ZINC SUPPLEMENTATION ON CADMIUM, ZINC AND COPPER IN LIVER, MUSCLE, HAIR, BLOOD AND FECES OF CALVES FED CADMIUM

The Ohio State University

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THE EFFECT OF ZINC SUPPLEMENTATION ON CADMIUM, ZINC
AND COPPER IN LIVER, MUSCLE, HAIR, BLOOD
AND FECES OF CALVES FED CADMIUM

DISSERTATION

Presented in Partial Fulfillment of the Requirement for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
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* * *

The Ohio State University
1981

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This work is dedicated to my wife, Avonelle, whose love and support sustained me; and to my sons, Daniel and Alan, who provided inspiration.
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FIELDS OF STUDY

Food Hygiene

Environmental Toxicology
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I. INTRODUCTION

Contamination of the environment by cadmium has increased in recent years as a result of its increased industrial use, its presence in agricultural fertilizer, and disposal of wastes containing cadmium (Friberg, et al., 1974; Luckey et al., 1975; Dorn, 1979). World production of cadmium has risen steadily and was greater than 31,000,000 pounds for the year 1972 (Fassett, 1972).

Metallic cadmium is extensively used in electroplating and alloys (Page, 1974) and cadmium compounds may be found in pigments, fungicides and plastics (MacGregor, 1975; Page, 1974). Little effort is made to recover cadmium from scrap processing and it is estimated that only 4% of new cadmium metal comes from scrap collection (MacGregor, 1975). Failure to recycle cadmium inevitably results in further environmental contamination.

Cadmium occurs naturally in sulfide deposits where it accompanies zinc, mercury, lead and copper in the ores of those metals (Fleischer et al., 1974). Zinc always occurs in some ratio with cadmium (MacGregor, 1975). Ratios of Zn:Cd in nature may vary as greatly as recorded extremes of 27 to 7,000 (Fleischer et al., 1974) but the most frequent is on the order of 100 (Fassett, 1975).

The cadmium content of the earth's crust is generally estimated to be 0.15 to 0.2 μg/g (Beliles, 1975; Fleischer et al., 1974) and soils...
can be expected to contain similar concentrations. Cadmium, although not essential to plant growth, is readily absorbed and is detectable in most plants (Hemphill, 1972).

Man-made circumstances have resulted in cadmium's accumulation in soils and subsequent translocation into human and animal foods. Water runoff from mine spoils in Japan produced toxic levels of cadmium in rice (Kobayashi, 1970). Copper smelters (Ratsch, 1974) and lead smelters (Hemphill et al., 1973) have caused increased cadmium concentrations to develop in adjacent soils.

The recent interest in applying municipal sewage sludges to agricultural land as a means of recycling plant nutrients and simultaneously disposing of the sludge (U.S. Environmental Protection Agency, 1976) creates the hazard of cadmium being translocated from sludge into the food chain (Pahren et al., 1977).

Corn grain grown on sludge amended soils was shown to translocate cadmium to pheasants fed the corn and result in increased liver and kidney cadmium concentrations (Melsted et al., 1977). Kienholz et al. (1976) reported increased liver and kidney cadmium content in cows grazing on a sludge recycling site.

The toxicity of cadmium has been well documented in animals and humans. Animals experimentally fed cadmium at moderately high levels usually show a decreased growth rate and decreased productivity. Doyle et al. (1974) reported decreased growth rates in lambs fed cadmium. Dietary cadmium caused depressed feed consumption and body weight gain in rats (Pond and Walker, 1975).
Young growing animals are more responsive to the toxic effects of cadmium than adult animals (Fox, 1976). Teratologic effects and increased mortality rates have been demonstrated in rat fetuses (Sonawane et al., 1975) and chick embryos (Birge and Roberts, 1976) as a result of cadmium exposure.

The nephrotoxic effects of cadmium in rats have been associated with cadmium–metallothionein induced necrosis of proximal renal tubular epithelium (Cherian et al., 1976; Itokawa et al., 1978). Koller and Brauner (1977) demonstrated a cadmium associated immunosuppression in the mouse.

In humans, cadmium has been associated with cardiovascular disease (Petit Clerc et al., 1977; Voors and Shuman, 1977), renal cancer (Kolonel, 1976), and prostatic cancer (Kipling and Waterhouse, 1967). Long term cadmium exposure causes emphysema (Friberg, 1950), renal tubular dysfunction (Bernard et al., 1976; Piscator and Pettersson, 1977) and osteomalacia (Kobayashi, 1971) in humans.

Cadmium and zinc interact metabolically and have been shown to be antagonistic in animals (Friberg et al., 1974) and in man (Suzuki et al., 1965; Tsuchiya and Iwao, 1978). The metabolism of both cadmium (Cherian et al., 1978; Nordberg and Nordberg, 1975; Webb, 1975) and zinc (Cousins, 1979; Johnson and Evans, 1980) are closely associated with low molecular weight proteins known as metallothioneins.

Nordberg and Nordberg (1975) identified a cadmium-thionein and a zinc-thionein in mouse liver and noted that each contained a minor amount of the opposite metal. Leber and Miya (1976) showed that
injected cadmium displaced zinc from both mouse liver cadmium- and zinc-thioneins.

Feeding high levels of cadmium produced an increased zinc content in calf kidney (Powell et al., 1964), calf liver (Miller et al., 1968) and lamb liver and kidney (Doyle and Pfander, 1975) as well as increased tissue cadmium contents in these animals. These experiments did not reveal the metabolic mechanisms through which zinc and cadmium apparently interacted to result in the reported zinc concentration increases.

Zinc is a metabolic antagonist of cadmium and tissue zinc levels are altered by cadmium administration. Zinc is also an essential trace element for man (Luckey et al., 1975) and most other life forms (Underwood, 1977). Therefore, the possibility of using supplemental zinc to influence tissue cadmium content, especially in food animals, is worthy of investigation.

The experiments of Powell et al. (1964) Miller et al. (1968) and Doyle and Pfander (1975) showed that tissue zinc concentrations increased in response to cadmium administration when the animals' feeds contained normal zinc levels. The possible effects on tissue cadmium of substantial, practical, non-toxic (Church, 1969) zinc supplementation, such as the 200 or 600 µg/g zinc levels evaluated in this study, are unknown in food animals.

The primary objective of this work is to evaluate the effect of practical levels of supplemental zinc on the cadmium content of edible
tissues (liver, kidney and muscle), hair and blood of calves fed cadmium.

The second objective is to develop a bovine model for predicting dietary cadmium and zinc levels from tissue, hair, blood and fecal cadmium and zinc concentrations.

The third objective arises from the opportunity to analyze the sample preparations for copper as well as for cadmium and zinc with a minimum of added expense. Copper is reported to interact with cadmium-thionein (Irons and Smith, 1976), to share metallothionein binding with zinc (Johnson and Evans, 1980) and to undergo altered metabolism in the presence of increased cadmium levels (Petering et al., 1979a) and increased zinc levels (Allen and Masters, 1980). Observation of tissue copper concentrations in relation to concentrations of cadmium and zinc may provide useful information on the practicality of feeding supplemental zinc and provide the opportunity to compare the findings of this study on calves with the findings of other studies. If the feeding of supplemental zinc adversely affected copper metabolism, its practical value would be diminished.
II. REVIEW OF THE LITERATURE

A. Absorption, Distribution and Excretion of Cadmium

Exposure to cadmium for both animals and man is primarily through ingestion of food and, to a much lesser extent, water which contain trace quantities of cadmium (Friberg et al., 1974). Consequently, a review of experimental work involving the oral administration of cadmium to animals or humans rather than parenteral administration more closely parallels natural cadmium exposure and absorption conditions.

Doyle et al. (1974) fed 4-month-old lambs 60 \( \mu g/g \) cadmium as \( \text{CdCl}_2 \) in their diet and found an apparent absorption of 5% of the ingested cadmium. Lactating Jersey cows retained about 0.75% of a single oral dose of \( \text{109CdCl}_2 \) after 14-16 days (Neathery et al., 1974). Thirty-four per cent of the retained \( \text{109Cd} \) was present in the gastrointestinal tract and its contents. Fourteen days following a single oral dose of \( \text{109Cd} \) 4- to 6-month-old goats showed a total body retention of 0.3% to 0.4% of the \( \text{109Cd} \) dose (Miller et al., 1969). Somewhat less than one fourth of this was in the gastrointestinal tissues and contents.

Sell (1975) concluded that the near absence of radioactivity in blood of hens given oral \( \text{109CdCl}_2 \) indicated poor absorption of cadmium. This indication was supported by the patterns of excretion in which approximately 70 to 80% of \( \text{109Cd} \) given appeared in the excreta.
during the first 24 hours after dosing. By 96 hours post dosing, 90 to 93% of the radioactivity had been excreted.

McLellan et al. (1978) conducted cadmium absorption studies on 14 healthy human volunteers. The subjects were fed 22-29 μg of $^{115}\text{mCd}$ as $^{115}\text{mCdCl}_2$. A whole body counter was used to determine the radioactivity from $^{115}\text{mCd}$ remaining in the subjects' bodies. Initial loss of radiocadmium was rapid with 86% of the dose being excreted in the first week. The average retention of radiocadmium was $4.6 \pm 4.0\%$ (SD) with a range of 0.7 to 15.6%.

Jacobs et al. (1978) fed Japanese quail low levels of dietary cadmium (0.02, 0.82, 0.145, 0.270, 0.520, and 1.02 μg/g) and found a linear log-dose, log-response accumulation of cadmium in the duodenum, liver and kidney. The proportion of cadmium retained by the liver and kidney was small compared to that of the duodenum. They further noted, that with those low dosage levels, the proportion of cadmium dose retained was independent of the dose level.

Neathery et al. (1974) studied distribution of a single oral dose of $^{109}\text{CdCl}_2$ in lactating Jersey cows. The cows were killed 14-16 days after dosing and the tissue and organ levels of $^{109}\text{Cd}$ were determined. Kidney contained the highest concentration of $^{109}\text{Cd}$ and was taken as a 100% level for comparison with other organs. Liver contained 49%, heart 1.3% and skeletal muscle 0.7% of the concentration of the kidney.
An experiment which produced cadmium tissue residues in swine was reported by Hammel et al. (1977) who fed sewage sludge, an important potential source of cadmium exposure in agriculture. Sewage sludge was fed as 0, 10, or 20% of the diet of 4-week-old weanling pigs. The pigs were fed to market weight and slaughtered. Analyses of their tissues revealed significantly (p < 0.05) higher liver and kidney cadmium contents in the 20% sludge-fed group.

Kienholz et al. (1976) compared the tissue cadmium levels of 12 old cows which had grazed for several years on municipal sewage sludge treated range land with the tissue cadmium levels of 6 control cows. The cadmium concentrations in both kidney and liver of sludge exposed cows were significantly (p < 0.05) higher than those of control cows.

Absorption of cadmium from the gastrointestinal tract apparently is greatest in the region of the duodenum. Jacobs et al. (1978) found that the duodenum of the Japanese quail maintained a cadmium concentration 16 times that of the diet and greater than other segments of the intestine.

Friberg et al. (1974) reviewed data on gastrointestinal absorption of cadmium and concluded that animals absorb about 2% of ingested cadmium with large individual variations and that humans have an average cadmium absorption of about 6%.

Cadmium crosses the placenta and accumulates in the fetus; however, the placenta does form a partial barrier to this transfer. Cadmium administered intravenously to rats crossed the placenta at all
dose rates and all gestational ages studied and accumulated in the fetus, primarily the liver (Sonawane et al., 1975). This study also showed an increasing placental cadmium concentration at older gestational age exposure times. Embryotoxicity and teratogenicity to rat fetuses were not seen with a maternal intravenous dose of 0.1 mg Cd/kg body weight but were observed after a 1.6 mg Cd/kg dose (Sonawane et al., 1975).

Excretion of cadmium is primarily in the feces. Neathery et al. (1974) found that cows given $^{109}$CdCl$_2$ orally excreted most of the $^{109}$Cd in the feces. High concentrations of injected $^{115}$mCd in the intestinal wall and contents of Japanese quail suggested that cadmium is excreted mainly into feces via the intestinal tract (Nishimura et al., 1974).

The biliary excretion of cadmium minus the portion reabsorbed contributes to fecal excretion. Stowe (1976) cannulated the bile ducts of rats before they were given subcutaneous doses of cadmium and then collected and analyzed bile for cadmium. The cadmium content of the bile was minimal within 3 hours of cadmium injection and it was estimated that 0.03 to 0.1% of the injected cadmium dose was excreted in the bile during the 5 hours of observation.

Stowe (1976) further reported that rats fed 100 $\mu$g/g cadmium as CdCl$_2$ for at least 21 days produced significantly more ($p < 0.01$) bile per unit body weight per hour than did other rats regardless of weight. He concluded that relatively small quantities of cadmium are excreted via the biliary route even following high dietary or parenteral
administration of cadmium. Doyle et al. (1974) observed that reexcretion of cadmium in the bile of lambs was negligible.

Urine is a minor route of excretion for ingested cadmium. Doyle et al. (1974) observed that urine cadmium excretion was negligible in lambs fed cadmium. Cows given oral $^{109}	ext{CdCl}_2$ excreted about 0.05% of the $^{109}	ext{Cd}$ dose in the urine within 14 days (Neathery et al., 1974).

B. Absorption, Distribution and Excretion of Zinc

The mechanism of zinc absorption and homeostatic control remains incompletely understood. This is particularly true regarding the generally accepted but poorly defined role of metallothionein as a binding, transporting and homeostatic medium within the intestinal mucosal cell (Cousins, 1979).

The general sites of zinc absorption and excretion and the overall tissue exchange dynamics of zinc have been more fully studied, especially in ruminants. Hiers et al. (1968) used a single oral dose of $^{65}	ext{Zn}$ to study absorption of zinc in different segments of the gastrointestinal tract of calves and goats. They found that $^{65}	ext{Zn}$ was secreted into the rumen and anterior section of the small intestine and reabsorbed in the abomasum and lower sections of the small intestine.

Pate et al. (1970) introduced $^{65}	ext{Zn}$ by catheter into the duodenum of calves and found that intestinal absorption of $^{65}	ext{Zn}$ was most rapid during the first hour and occurred almost entirely above the level of the cecum.
Intestinal metallothionein is inducible by zinc and is commonly designated zinc-metallothionein since it is thought to also be capable of binding copper and cadmium (Cousins, 1979).

Starcher et al. (1980) studied the zinc induction of intestinal metallothionein and concluded that zinc absorption was directly proportional to intestinal metallothionein levels. This is not in agreement with unpublished data of Cousins (1979) which indicated that intestinal metallothionein was inversely related to the absorption process in that it acted as a controlling influence on the otherwise direct transfer of zinc across intestinal mucosal cells into plasma.

Oh et al. (1979) eluted the cytosols of various tissues from chicks fed 500 to 4,000 μg/g zinc through G-75 Sephadex columns. For each tissue, zinc was associated with 4 peaks, one of which was metallothionein. The tissue metallothioneins increased in response to increased dietary levels of zinc more than the other 3 fractions. The zinc content of tissue metallothioneins decreased rapidly when chicks were removed from high zinc feeding levels to normal feeding levels indicating an involvement of metallothionein in tissue zinc homeostasis and an extreme lability of zinc in metallothionein.

Huber and Gershoff (1970) found the rate of retention of both oral and intraperitoneally injected 65Zn was greater in rats receiving low rather than high levels of dietary zinc. This was evidence that a homeostatic mechanism was operative regardless of the route of 65Zn administration.
Miller et al. (1970) fed calves 0, 200 and 600 µg/g supplemental zinc then dosed them once with $^{65}$Zn. They observed decreased liver, kidney and blood $^{65}$Zn in calves receiving 200 µg/g zinc and increased $^{65}$Zn in pancreas, kidney and liver of calves receiving 600 µg/g zinc. They concluded that a homeostatic mechanism operated to exclude additional zinc at the 200 µg/g feeding level but that homeostasis was overcome at the 600 µg/g feeding level.

Ott et al. (1966b) also observed that the zinc absorption control mechanism in lambs was altered at extremely high (2,000-4,000 µg/g) dietary zinc levels resulting in much increased zinc concentrations in serum, pancreas, liver and kidney.

Several investigators have studied organ distribution and resulting tissue zinc levels from high dietary zinc. In calves fed 200 µg/g added zinc, the zinc concentration was moderately increased in liver and pancreas, slightly increased in kidney, blood and hair and unchanged in muscle and bone (Miller et al., 1970).

Miller et al. (1970) concluded from zinc feeding studies in calves that muscle has a given number of zinc binding sites which are always completely saturated and hold zinc with great tenacity; whereas liver and pancreas have several types of zinc binding sites which hold zinc with varying degrees of tenacity.

Ott et al. (1966b) fed lambs 0, 500, 1,000, 2,000 and 4,000 µg/g added zinc for 6 to 10 weeks and observed liver zinc concentrations of 35, 38, 91, 427 and 398 µg/g for the respective feeding levels. These
data indicate very little increase in liver zinc concentration from 500 µg/g zinc supplementation.

Feeder calves were fed between 100 and 2,100 µg/g of dietary zinc (as ZnO) for 12 weeks (Ott et al., 1966c). Kidney zinc concentrations were most increased, followed closely by that of liver. Hair zinc concentrations increased slightly while muscle zinc was unchanged.

Similar results were achieved in another experiment with lambs fed 2,000 µg/g zinc for 6 to 10 weeks. They had a kidney zinc concentration of 448 µg/g compared to 17 µg/g for control lambs while muscle and wool zinc concentrations remained unchanged (Ott et al., 1966b).

Lactating cows fed 0, 500, 1,000 and 2,000 µg/g added zinc as ZnO for 6 weeks showed increased blood plasma and milk zinc concentrations but no change in production, body weight or feed consumption (Miller et al., 1965).

Normal dietary zinc levels of 18-50 µg/g (Church, 1969) must be exceeded many times to approach toxic levels for ruminants. Ott et al. (1966b) observed altered rumen digestion which resulted in impaired digestion and reduced feed efficiency and in connective tissue replacement of pancreatic acinar tissue in lambs at dietary zinc levels over 1,000 µg/g. Lambs showed reduced gains when fed 1,000 µg/g of added dietary zinc but not when fed 500 µg/g (Ott et al., 1966a).

Investigators feeding supplemental zinc have used various chemical forms of zinc including zinc oxide (ZnO) (Miller et al., 1965;
Ott et al., 1966c), zinc chloride (ZnCl₂) (Huber and Gershoff, 1970) and zinc sulfate (ZnSO₄·7 H₂O) (Ott et al., 1966b). Miller et al. (1970) found that ZnO and ZnSO₄ gave comparable results in raising tissue zinc levels when fed to calves.

In reviewing zinc excretion data for ruminants, Church (1969) found that feces was the major route of excretion and that urinary excretion was generally reported to be less than 1% of ingested zinc. Rats given both oral and intraperitoneal ⁶₅Zn excreted it mostly in the feces while less than 1% was excreted in urine (Huber and Gershoff, 1970).

Miller et al. (1970) found urine zinc excretion declined as a percentage of dietary intake when calves were fed supplemental zinc and that the amount of zinc excreted in urine was nominal compared with fecal zinc excretion.

C. Interactions of Cadmium and Zinc

Absorbed cadmium is quickly bound by proteins. Specific, cadmium inducible, low molecular weight apoproteins called thioneins bind cadmium primarily in the liver (Nordberg and Nordberg, 1975; Webb, 1975). Shaikh and Lucis (1972) have suggested that intracellular cadmium-thionein is a biochemical mechanism for sequestering toxic Cd²⁺ ions in mammals.

The metabolism of zinc is also closely associated with metallothioneins. Subcutaneously injected ¹⁰⁹Cd and ⁶₅Zn which
concentrated primarily in liver and kidney of rats were bound to proteins (Shaikh and Lucis, 1972).

Metallothioneins have been isolated from liver and other tissues of many species of animals including man. A cadmium-binding protein has been identified in the liver of humans, monkeys (Shaikh and Lucis, 1972), rabbits and mice (Nordberg, 1978) and in the liver and kidney of rats (Shaikh and Smith, 1976), cows, swine and chickens (Verma et al., 1978). Webb (1975) reported zinc-thionein present in low concentration in the testis of the male rat. The liver of the pig is capable of synthesizing zinc-thionein (Daniel et al., 1977).

Specific metallothioneins have a predominant affinity for either cadmium or zinc and several investigators have observed displacement of one metal by the other. Two fractions of liver metallothionein were identified by Nordberg and Nordberg (1975) in the mouse. The first metalloprotein fraction was found to consist of 32% cysteine residues and have no aromatic amino acids. It contained 61% of the total cadmium but very little zinc and was designated MT1. The second metallothionein, MT2, contained 24% of the total cadmium and the main part of the zinc. The cadmium-MT1 complex was selectively accumulated by the kidney and a portion of it was excreted in the urine.

Leber and Miya (1976) also demonstrated a zinc-binding protein and a cadmium and zinc-binding protein in livers of mice. Cadmium injected subcutaneously displaced zinc from both these proteins in vivo.
Both cadmium and zinc were bound to newly synthesized liver metallothionein in nearly equal quantities in rats (Cempel and Webb, 1976). When additional cadmium was injected, zinc was displaced by cadmium. Zinc was again incorporated to a nearly equal extent with cadmium as additional thionein was synthesized in response to the newly injected cadmium. Parenterally administered cadmium caused systemic mobilization of zinc in rats and induced liver thioneins which bound both cadmium and zinc (Winge et al., 1978).

Both cadmium and zinc are known to induce synthesis of metallothionein. Metallothionein induced in livers of rats and mice by cadmium injection contained 2 zinc-binding sites for every 8 possible sites (Winge et al., 1978). Metallothionein has been induced in liver and kidney by parenteral cadmium in rabbits and mice (Nordberg, 1978) and by oral cadmium (2 and 10 µg/g) in cows, swine and chickens (Verma et al., 1978).

Webb (1972) demonstrated zinc induction of metallothionein in rats which protected against cadmium toxicity. Zinc (0.1 to 0.5 mmole/kg body weight) injected 24 hours previously protected mice against the lethality of a subcutaneous injection of 0.038 mmole CdCl₂/kg body weight (Gunn et al., 1968). Zinc-thionein however, did not protect rats from cadmium toxicity in a study by Webb and Verschoyle (1976).

Webb and Verschoyle (1976) reported that cadmium was more strongly bound to rat metallothionein than was zinc. Kagi and Vallee (1961) found that cadmium bound 3,000 times more firmly than zinc to equine
metallothionein. Cadmium-thionein had a half-life up to twice as long (3.3 days in rat liver and 3.6 days in rat kidney) as zinc-thionein (Ohanian et al., 1978).

Cadmium-zinc interactions have been observed by Powell et al. (1964), Doyle and Pfander (1975) and Miller et al. (1968) during cadmium feeding experiments with ruminants. Powell et al. (1964) reported an increased kidney zinc concentration and a decreased blood zinc level in calves fed 640 and 2,560 µg/g cadmium without zinc supplementation.

Doyle and Pfander (1975) fed lambs diets containing 0, 5, 15, 30, and 60 µg/g cadmium but no added zinc for 191 days and produced marked increases in tissue levels of cadmium. The zinc content of liver and kidney was increased but that of heart, lung, and spleen remained unchanged. A cadmium-zinc interaction occurred as zinc increased in association with increased cadmium in liver and kidney.

Miller et al. (1968) also observed an increase in the zinc content of livers when calves were fed 350 µg/g cadmium. They observed no consistent effect on the zinc content of heart, lung, kidney, bone or muscle from feeding cadmium to zinc-deficient and to normal calves but did note that feeding cadmium increased fecal excretion of zinc.

Similar interactions have been reported in rats and chicks. Pond and Walker (1975) reported that feeding rats high dietary cadmium (200 µg/g) significantly (p < 0.01) increased liver zinc and decreased liver iron concentrations. The liver zinc concentration of two-week-
old chicks was increased 80% and kidney zinc increased 44% by feeding them a diet of 700 µg/g cadmium (Pritzl et al., 1974).

Long-term retention of \(^{115}\text{mCd}\) was decreased in Japanese quail by supplemental feeding of zinc, manganese and copper. Jacobs et al. (1978) demonstrated that a ration which was moderately supplemented to levels of 30 µg/g Zn, 5 µg/g Cu and 12 µg/g Mn reduced the accumulation of dietary cadmium in liver and kidney but not in duodenum of Japanese quail. It could not be determined from this experiment whether zinc, copper, manganese or a combination of them was responsible for the effect.

Elinder and Piscator (1978) plotted the increases observed for zinc in association with experimentally increased cadmium in kidney cortex and liver of various species. Humans and large farm animals accumulated zinc in kidney cortex in a nearly equimolar ratio with cadmium (Zn: Cd of 0.6 to 1.0). Smaller laboratory animals, rats, mice, guinea pigs and rabbits, had incremental zinc to cadmium ratios of 0.0 to 0.5. When incremental zinc to cadmium ratios were plotted for liver, a reverse species effect was noted with large farm animals (lambs and pigs) having a zinc to cadmium ratio of less than 0.5 while laboratory animals' ratios were 0.6 to 2.0. Zinc to cadmium ratios of large farm animals were similar to those of man and markedly different from those of the smaller laboratory animals.

D. Interactions of Copper with Cadmium and Zinc

Copper is known to have metabolic interactions with both cadmium (Petering et al., 1979b) and zinc (Allen and Masters, 1980). The
mechanisms of copper, cadmium and zinc interactions are not well understood but there is evidence that metallothioneins play a partial role. Suzuki and Yamamura (1981) detected copper in the 3 metallothionein fractions of normal rat kidneys. These fractions also contained zinc and were the fractions in which cadmium appeared after it was administered to the animals.

An indication of the ability of copper to interfere with cadmium metabolism is evidenced by the finding of Irons and Smith (1976) that copper caused a failure of cadmium sequestration by metallothionein in the livers of rats administered high doses of cadmium and copper salts.

Although a simple interference in absorption may occur in which copper, zinc and cadmium as relatively similar divalent cations may compete for mucosal binding sites or tend to influence the relative solubilities of each other, no evidence is available that competition for active transport is a factor. Adham and Song (1980) noted no effect of excess cupric ions on the amount of $^{65}$Zn absorbed from the small intestines of rats and concluded that zinc absorption in rats is mediated by a transcellular transport process different from that which mediates copper absorption.

Increased kidney copper concentrations have been associated with increased cadmium body burdens in rats and lambs. Stonard and Webb (1976) reported an increase in thionein-bound copper in the kidneys of rats fed 100 $\mu$g/g cadmium for 40 weeks. In an experiment reported by Suzuki and Yamamura (1980), the copper concentration of rat kidney
decreased for the first 3 days after intraperitoneal injection of CdCl$_2$ and then returned to the pre-injection level at 7 days. During repeated subcutaneous injections of CdCl$_2$, the kidney copper concentrations of rats decreased for the first 3 weeks but then increased slightly over pre-injection levels after 4 weeks (Suzuki et al., 1980).

Rats administered cadmium in drinking water for 39 weeks had increased kidney copper concentrations (Petering et al., 1979b). Julshamn et al. (1977) reported no change in liver copper concentrations but an increase in kidney copper concentration in rats fed 100 µg/g cadmium for 9 weeks. Lambs fed 30 and 60 µg/g cadmium for 191 days showed increased kidney copper concentrations (Doyle and Pfander, 1975).

In contrast to the association of cadmium and increased kidney copper concentrations in rats and lambs, Nordberg et al. (1979) analyzed kidneys of 20 normal horses and found that copper concentrations were not related to cadmium concentrations. Dorn et al. (1974b) reported lower blood, kidney, liver and muscle copper concentrations in cows exposed to high environmental levels of lead, zinc, cadmium and copper.

Petering et al. (1979a) found that copper metabolism was disturbed in female rats receiving 17.2 µg/ml cadmium in drinking water resulting in lowered whole body copper concentrations in pups born to these mothers.
Zinc appears to have a depressing effect on liver copper concentrations. Liver copper concentration was decreased in sheep treated orally for 6 days with 2 g of zinc sulfate/day (Allen and Masters, 1980). When zinc was fed to calves at 900 μg/g (Ott et al., 1966c) and to lambs at 2,000 μg/g (Ott et al., 1966b), liver copper concentrations were decreased.
III. MATERIALS AND METHODS

A. Experimental Design

The experiment consisted of 3 groups of 8 calves each which were fed rations to which had been added either cadmium only (zinc present at low, basal level); cadmium plus medium level zinc; or cadmium plus high level zinc and a fourth, baseline group of 8 calves.

One of the 4 calves from each of the 8 farms was randomly allocated to each one of the 4 experimental groups (Table 1) by use of random number tables (Dixon and Massey, 1969, pp 446-448).

Baseline group calves were slaughtered and sampled 3 days after delivery from farms and were not fed an experimental ration. Hair, liver, kidney and muscle samples from baseline calves represented the levels of cadmium, zinc and copper acquired from the environment of the farm of origin and provided an example of levels which could be regarded as normal.

The other 3 groups were fed experimental rations for 60 days and then slaughtered for final sampling. Table 2 gives the experimental ration fed to each treatment group. Group 1 (cadmium with low level zinc) was fed a basal ration with 50 µg/g added cadmium. Rations fed groups 2 and 3 also contained 50 µg/g added cadmium with the addition of 200 µg/g zinc for group 2 (cadmium plus medium level zinc) and 600 µg/g zinc for group 3 (cadmium plus high level zinc).
<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Total</th>
<th>Sludge Receiving Farms</th>
<th>Control Farms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>32</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Baseline</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

a One calf from each farm was randomly allocated to each treatment group.

b Baseline calves were not fed experimental rations and were sampled when received from farms. Group 1, 50 µg/g cadmium; group 2, 50 µg/g cadmium plus 200 µg/g zinc; and group 3, 50 µg/g cadmium plus 600 µg/g zinc added to ration for 60 days.
<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment, µg/g</th>
<th>Days on Feed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cd</td>
<td>Zn</td>
</tr>
<tr>
<td>Baseline&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 Low level zinc</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>2 Medium level zinc</td>
<td>50</td>
<td>200</td>
</tr>
<tr>
<td>3 High level zinc</td>
<td>50</td>
<td>600</td>
</tr>
</tbody>
</table>

<sup>a</sup> Baseline calves were fed only hay and water until they were slaughtered and sampled 3 days after being received from farms.
Calves were received from the farms at 4 different times, October 22, 1979, November 26, 1979, January 14, 1980, and February 11, 1980. Calves from 2 farms were received at each time. After calves were adjusted to the pelleted basal ration, experimental rations were started and fed continuously, twice a day, for 60 days. Cohort lots of calves were started on experimental rations November 11, 1979, December 11, 1979, January 28, 1980, and February 18, 1980.

Samples of liver, kidney, muscle, hair, blood and feces were collected from calves to be analyzed for cadmium, zinc and copper by atomic absorption spectroscopy. Samples of calf blood and feces were collected prior to (prefeeding or 0 days) and at 0.5, 1.5, 3.5, 8, 15, 30, 45, and 60 days after starting to feed experimental rations.

Hair samples were taken on the day calves were received from the farms and again at the end of the 60 day experimental feeding period.

Calves were slaughtered at the end of the 60 day feeding period for final tissue (liver, kidney and muscle) sampling. Slaughter dates for experimentally fed calves were January 10, 1980, February 11, 1980, March 31, 1980, and April 21, 1980. A sampling schedule for the experiment is presented in Table 3.

B. Animals

Eight farms which were participating in a demonstration project for the practical utilization of municipal sewage sludge as a pasture fertilizer each provided 4 calves to the experiment. Calves were of mixed beef breeding, weighed between 85 and 283 kg (mean, 184 kg) and were from 6 to 9 months of age when they entered the experiment.
### TABLE 3

Sample Collections from Treatment Groups 1, 2, and 3 Calves for Cadmium, Zinc and Copper Analyses by Time and by Sample Type

<table>
<thead>
<tr>
<th>Time of Collection&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Liver</th>
<th>Kidney</th>
<th>Muscle</th>
<th>Hair</th>
<th>Blood</th>
<th>Feces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Received from Farms&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 Days (Pre-feeding)</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 Days</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5 Days</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.5 Days</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 Days</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 Days</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 Days</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45 Days</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 Days</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup> The reference point for time of collection is the start of feeding of the experimental rations containing added cadmium or cadmium and zinc.

<sup>b</sup> Baseline group calves were also sampled (liver, kidney, muscle, and hair) at the time received from farms.
Calves from the 4 demonstration control farms had no exposure to sewage sludge whereas the calves from the 4 sludge receiving farms had low level exposure to cadmium from the sludge treated pastures upon which they and their dams were grazed. On the day calves were received from farms, they were weighed; hair, blood and feces samples were taken; and those in groups 1, 2, and 3 were vaccinated against infectious bovine rhinotracheitis and parainfluenza 3 (Nasalgen, Jensen-Salsbery Laboratories, Kansas City, MO).

Baseline group calves were allowed free access to hay and water until they were slaughtered. Calves in groups 1, 2, and 3 were provided with water ad libitum throughout the feeding period. Calves were adjusted to the complete pelleted experimental ration over a period of from one to 3 weeks. They were fed all the good quality mixed grass-legume hay they would eat for the first 2 days; and then the hay was gradually decreased while the pelleted basal ration without cadmium or zinc was introduced and increased. Calves were started on the appropriate cadmium or cadmium plus zinc experimental ration when all were consuming 1% of their body weight of basal ration twice a day.

Calves were maintained in rubber surfaced tie stalls without bedding in an enclosed cattle facility of The Ohio State University, College of Veterinary Medicine, from the time they were received from farms of origin until they were slaughtered for final sampling.

C. Rations

Rations were prepared at the Ohio Agricultural Research and Development Center (OARDC) feed mill at Wooster, Ohio. This mill was
designed for formulating experimental rations and had specialized equipment and trained personnel for that purpose.

A modified formulation of a pelleted complete ration known as OARDC Heifer Ration D-433-78 was used as the basal ration. The formulation of this ration which contained 48% corn cobs as a roughage base along with oats, alfalfa meal, soy bran flakes and mineral and vitamin supplements is given in Table 4.

The first batch of rations was prepared November 2, 1979, and the second batch February 2, 1980. All rations prepared in the first batch were derived from the same lots of basic ingredients. Sufficient quantities of sulfate supplement, trace mineral salt, sodium bentonite and limestone were retained to be used in formulation of the second batch.

Major ingredients and those minor ingredients, with the exception of those retained as stated above, used for the second batch were from different lots than those used in the first batch. A quantity of the basal ration without added cadmium or zinc was prepared in the first batch to be used to accustom all lots of calves to the all-pelleted complete ration.

Each ration was weighed out and mixed separately. These 4 rations were prepared in this order: basal ration (no added Cd or Zn); ration 1, basal ration with 92.15 g CdCl₂ · 2-1/2H₂O added/ton (50 µg Cd/g ration); ration 2, basal ration with 92.15 g CdCl₂ · 2-1/2H₂O and 225.81 g ZnO added/ton (50 µg Cd and 200 µg Zn/g ration); and ration 3,
TABLE 4

Formulation of Complete Pelleted Basal Ration*

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>100.00%</td>
</tr>
<tr>
<td>Corn cobs (No. 4), ground</td>
<td>47.97</td>
</tr>
<tr>
<td>Oats, ground</td>
<td>19.00</td>
</tr>
<tr>
<td>Alfalfa meal</td>
<td>10.23</td>
</tr>
<tr>
<td>Soy bran flakes</td>
<td>10.00</td>
</tr>
<tr>
<td>Urea</td>
<td>1.00</td>
</tr>
<tr>
<td>Whey</td>
<td>3.00</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.26</td>
</tr>
<tr>
<td>Salt, trace mineral</td>
<td>0.40</td>
</tr>
<tr>
<td>Sulfate supplement ($K_2SO_4, MgSO_4$)</td>
<td>0.21</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>0.067</td>
</tr>
<tr>
<td>Vitamin D-3</td>
<td>0.033</td>
</tr>
<tr>
<td>Sodium bentonite</td>
<td>2.00</td>
</tr>
<tr>
<td>Molasses, dried</td>
<td>2.83</td>
</tr>
<tr>
<td>Animal-vegetable fat</td>
<td>2.00</td>
</tr>
</tbody>
</table>

*Ohio Agricultural Research and Development Center, Heifer Ration D-433-78 (Modified)
92.15 g CdCl$_2$ $\cdot$ 2-1/2H$_2$O and 677.43 g ZnO added/ton (50 $\mu$g Cd and 600 $\mu$g Zn/g ration).

The actual cadmium, zinc and copper concentrations of the finished rations, as determined by analysis, are shown in Table 5. The mixer was thoroughly swept out between rations.

The major ingredients were added first to a 3,000 lb. capacity horizontal ribbon-type mixer. Next the minor ingredients were added and finally a portion of the ground oats to which had been added the experimental elements. Reagent grade CdCl$_2$ $\cdot$ 2-1/2H$_2$O as required for each ration was dissolved in 400 ml of 0.0025% HNO$_3$. The CdCl$_2$ solution was then poured slowly into the hollowed out center of an approximately 35 lb. portion of ground oats which was contained in the bowl of a Hobart model D-300T eccentric bowl-type rotary premixer. The oats and CdCl$_2$ solution were mixed for 12 minutes.

For rations requiring zinc addition, the proper quantity of dry powdered reagent grade ZnO was added to the ground oats first and mixed for 2 minutes before the CdCl$_2$ solution was added. The resulting oats with cadmium or cadmium and zinc premix was then added to the other ingredients already in the ribbon mixer and all were mixed for 10 minutes.

Once calves were started on the experimental rations, they were fed only the experimental rations in an amount equal to 1% of their initial body weights twice a day continuously for 60 days. An exception was calf number 55 which was fed approximately 0.5 lb. of hay
TABLE 5  

Cadmium, Zinc and Copper Concentrations\(^a\) of Experimental Rations by Batch and by Treatment Group

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Cd/Zn Additions</th>
<th>Batch No.  (Date Mixed)</th>
<th>Concentration ((\mu g/g)) in Ration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cd Zn Cu</td>
<td></td>
<td>Cd Zn Cu</td>
</tr>
<tr>
<td>1</td>
<td>50 (\mu g) Cd/g</td>
<td>1-(11/2/79)</td>
<td>41.5 59 7.00</td>
</tr>
<tr>
<td>2</td>
<td>50 (\mu g) Cd/g</td>
<td>1-(11/2/79)</td>
<td>47.9 265 7.16</td>
</tr>
<tr>
<td></td>
<td>200 (\mu g) Zn/g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>50 (\mu g) Cd/g</td>
<td>1-(11/2/79)</td>
<td>52.9 709 7.31</td>
</tr>
<tr>
<td></td>
<td>600 (\mu g) Zn/g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>50 (\mu g) Cd/g</td>
<td>2-(2/7/80)</td>
<td>50.6 54 7.44</td>
</tr>
<tr>
<td>2</td>
<td>50 (\mu g) Cd/g</td>
<td>2-(2/7/80)</td>
<td>52.4 254 7.40</td>
</tr>
<tr>
<td></td>
<td>200 (\mu g) Zn/g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>50 (\mu g) Cd/g</td>
<td>2-(2/7/80)</td>
<td>49.3 641 7.34</td>
</tr>
<tr>
<td></td>
<td>600 (\mu g) Zn/g</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(\mu g/g\): micrograms per gram.

\(a\) Means of triplicate analyses.

\(b\) A basal ration without added cadmium or zinc was prepared 11/2/79 in batch no. 1 which contained 0.13 \(\mu g/g\) Cd, 69 \(\mu g/g\) Zn and 8.54 \(\mu g/g\) Cu.
per day (total 25 lb.) for 50 days of the feeding period as a bloat prevention measure.

The calves usually consumed their rations completely in less than 10 minutes except for infrequent instances when calves were off feed because of pneumonia, another health problem or an inapparent reason. Quantities of ration fed daily, ration fed but not consumed and ration actually consumed over 60 days for each calf are given in Table 6.

D. Sampling

Calves were slaughtered for sampling at the Meat Laboratory of The Ohio State University, Department of Animal Science. Approximately 0.25 kg of the caudal portion of the liver, the caudal half of the right kidney and approximately 0.25 kg of the right triceps muscle were collected.

Care was taken to avoid contamination of tissue with feces or tap water. Tissue samples were collected, frozen and stored in sealed polyethylene bags (Whirlpak, Nasco, Fort Atkinson, WI).

A hair sample (approximately 5 g) was clipped, using a number 10 blade, from the right mid-thoracic area of each calf when it was received from the farm. At the end of the 60 day experimental feeding period, the same area was again clipped in an effort to obtain a sample of hair regrowth occurring during this period. Another 60 day hair sample was taken from the corresponding area of the calf's left side for comparison purposes.

Hair samples were collected and stored in sealed polyethylene bags (Whirlpak, Nasco, Fort Atkinson, WI). Prior to analysis, hair samples
TABLE 6

Body Weights of Calves, Daily and 60 Day Ration Allowances, Ration Not Consumed and Actual Ration Consumed by Treatment Group and by Calf Number

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Calf No.</th>
<th>Body Weight</th>
<th>Daily Ration</th>
<th>60 Day Ration Allowed</th>
<th>Ration Not Consumed&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Actual Ration Consumed&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>45</td>
<td>249</td>
<td>5.0</td>
<td>299</td>
<td>17 (5.7)</td>
<td>282 (94.3)</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>196</td>
<td>3.9</td>
<td>235</td>
<td>0</td>
<td>235</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>185</td>
<td>3.7</td>
<td>222</td>
<td>3 (1.4)</td>
<td>219 (92.6)</td>
</tr>
<tr>
<td></td>
<td>54</td>
<td>151</td>
<td>3.0</td>
<td>181</td>
<td>0</td>
<td>181</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>244</td>
<td>4.9</td>
<td>292</td>
<td>2 (0.7)</td>
<td>290 (99.3)</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>185</td>
<td>3.7</td>
<td>222</td>
<td>7 (3.2)</td>
<td>215 (96.8)</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>89</td>
<td>1.8</td>
<td>107</td>
<td>1 (0.9)</td>
<td>106 (99.1)</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>283</td>
<td>5.7</td>
<td>340</td>
<td>0</td>
<td>340</td>
</tr>
<tr>
<td>Group 2</td>
<td>43</td>
<td>140</td>
<td>2.8</td>
<td>168</td>
<td>4 (2.4)</td>
<td>164 (97.6)</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>239</td>
<td>4.8</td>
<td>287</td>
<td>0</td>
<td>287</td>
</tr>
<tr>
<td></td>
<td>62</td>
<td>153</td>
<td>3.1</td>
<td>183</td>
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<td>183</td>
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<tr>
<td></td>
<td>51</td>
<td>195</td>
<td>3.9</td>
<td>234</td>
<td>0</td>
<td>234</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>248</td>
<td>5.0</td>
<td>298</td>
<td>4 (1.3)</td>
<td>294 (98.7)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>149</td>
<td>3.0</td>
<td>179</td>
<td>0</td>
<td>179</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>95</td>
<td>1.9</td>
<td>114</td>
<td>10 (8.8)</td>
<td>104 (91.2)</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>181</td>
<td>3.6</td>
<td>218</td>
<td>0</td>
<td>218</td>
</tr>
<tr>
<td>Group 3</td>
<td>44</td>
<td>171</td>
<td>3.4</td>
<td>205</td>
<td>0</td>
<td>205</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>189</td>
<td>3.8</td>
<td>227</td>
<td>16 (7.0)</td>
<td>211 (93.0)</td>
</tr>
<tr>
<td></td>
<td>61</td>
<td>137</td>
<td>2.7</td>
<td>164</td>
<td>0</td>
<td>164</td>
</tr>
<tr>
<td></td>
<td>53</td>
<td>161</td>
<td>3.2</td>
<td>193</td>
<td>0</td>
<td>193</td>
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<tr>
<td></td>
<td>40</td>
<td>244</td>
<td>4.9</td>
<td>292</td>
<td>0</td>
<td>292</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>123</td>
<td>2.5</td>
<td>148</td>
<td>0</td>
<td>148</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>117</td>
<td>2.3</td>
<td>140</td>
<td>0</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>63</td>
<td>199</td>
<td>4.0</td>
<td>239</td>
<td>0</td>
<td>239</td>
</tr>
</tbody>
</table>

<sup>a</sup> Group 1, 50 μg/g cadmium; group 2, 50 μg/g cadmium plus 200 μg/g zinc; group 3, 50 μg/g cadmium plus 600 μg/g zinc added to ration for 60 days.

<sup>b</sup> Ration not consumed is expressed in kg (per cent of 60 day ration allowed which was not consumed).

<sup>c</sup> Actual ration consumed is expressed in kg (per cent of 60 day ration allowed which was consumed).
were washed twice in a 1% solution of a low metal content surfactant (Snoop, Nupro Co., Willoughby, OH) and then rinsed 3 times with filter purified water and allowed to air dry.

Blood was collected by jugular venipuncture with a 1.5 inch, 14 gauge disposable needle. A stream of blood was allowed to drain freely from the needle into a narrow-mouthed 4 oz. polyethylene bottle (Nalge Co., Division of Sybron Corp., Rochester, NY). Blood collection bottles each contained 5,000 units (0.5 ml) of sodium heparin (Lipo-Hepin, Riker Laboratories, Inc., Northridge, CA) as an anticoagulant. Blood was kept frozen until analyzed.

Feces were collected in waxed paper fecal cups with plastic lids and stored at 4°C for up to 3 days before being dried for storage. Approximately 60 g of wet feces was spread 1 cm thick in aluminum foil pans and dried overnight at 105°C in a convection-type drying oven. Dried feces were then coarsely ground in nitric acid-washed porcelain mortars and stored in sealed 1 oz. polyethylene bottles (Nalge Co., Division of Sybron Corp., Rochester, NY).

E. Atomic Absorption Spectroscopy Analysis

General

Analyses were done on a double beam atomic absorption spectrophotometer (Model AA-775, Varian Techtron Pty. Ltd., Springvale, Australia) equipped with a deuterium lamp for simultaneous background correction for nonatomic absorption. Both flame (air-acetylene) and carbon furnace (Varian Model CRA-90 equipped with ASD-53 auto-sampler)
methods of sample atomization were employed depending on the concentration of analyte element present in the digested sample and the detection limits of the instrument.

Filter-purified water of 18 megaohm conductivity (Milli-Q Model MQ2, Millipore Corp., Bedford, MA) was used exclusively throughout the experiment for final glassware rinsing, sample processing and reagent preparation.

Laboratory glassware was washed 3 times with a caustic laboratory detergent (Event, Economics Laboratory, Inc., St. Paul, MN) and rinsed with distilled water in a mechanical dishwasher. Washed glassware and new sample containers were rinsed 3 times in filter-purified water, soaked overnight in 10% reagent grade nitric acid and given 3 final rinses with filter-purified water before being oven dried (glassware) or air dried (plastic-ware). Polyethylene film was wrapped around glassware before storage to prevent dustfall contamination.

Duplicate portions of each sample were measured out and taken through all stages of sample preparation and analysis except for muscle samples analyzed for zinc which were done singly. Tissues were cut with nitric acid rinsed stainless steel scalpels and all solid samples were handled, when necessary, with nitric acid rinsed plastic tipped forceps.

All samples were wet-digested in hot concentrated redistilled nitric acid (G.F. Smith Chemical Co., Columbus, OH) with 30% reagent grade \( \text{H}_2\text{O}_2 \) employed as an oxidation aid. Samples were either weighed (feces, hair, tissues) or pipetted (blood) into 30 ml Kjeldahl flasks.
Nitric acid was added and the samples were allowed to begin digestion slowly at room temperature, usually overnight, to control foaming. After nitric acid addition, samples were kept in a fume hood because of the almost immediate evolution of toxic brown oxides of nitrogen. When samples had become nearly solubilized and had passed through the foaming stage, they were placed into aluminum heating blocks and brought to a gentle boil (120-130° C).

Hydrogen peroxide was added at varying times during the boiling period. Samples were cooled prior to addition of H₂O₂, to control the potentially explosive evolution of oxygen which could occur from hot mixtures of nitric acid and H₂O₂. After the addition of H₂O₂, samples were continued on heat at a gentle boil for an additional 12 to 48 hours until solubilization was as complete as could be attained.

Samples which were to be highly diluted were removed from heat at this point. Those samples which could tolerate little dilution because of detection limit restraints were heated at higher temperatures (180-200° C) sufficient to evaporate nitric acid. Evaporation continued until the sample volume was reduced to between 2 ml and near dryness depending on the degree of dilution of nitric acid required for analysis.

Samples were quantitatively transferred from the Kjeldahl flasks to volumetric flasks and brought up to known volume before being stored in sealed 1 oz. polyethylene bottles (Nalge Co., Division of Sybron Corp., Rochester, NY) until analyzed.
The method of standard additions was used to calibrate atomic absorption readings for each sample type. Replicate flasks of a representative sample were measured out and spiked with known concentrations of the analyte element. Duplicate spikes of 0%, 50%, 100% and 150% of the sample's expected content of analyte element were prepared for a standard additions series. The standard additions series samples were digested, diluted and analyzed along with the other samples of a similar kind.

The absorbances of the standard additions series were plotted against the concentration of analyte element in the spike (Figure 1). The slope of the regression line of best fit was used to calculate the concentration of analyte element in other samples of the same kind from their absorbance readings. The concentration of analyte element was equal to the absorbance divided by the slope.

Detection limits were defined as sample analyte element concentrations equal to twice the standard deviation for replicate absorbance readings of a low concentration sample. Standard deviations for detection limit determinations were calculated from series of 10 repeated absorbance readings of relatively low concentration samples taken against the samples' blanks.

Detection limits for each sample type are given in Table 7. Analytical results were calculated and recorded as μg/100 ml for blood and as μg/g either on a wet basis for liver, kidney cotex and muscle or on a dry weight basis for hair, feces and rations.
Absorbances of a standard additions series of samples spiked at 0%, 50%, 100% and 150% of the expected sample analyte content are plotted against the spike concentration in the sample. The slope of the plotted standard additions curve is then used to calculate analyte concentration in other samples of similar matrix composition.
Absorbance

Concentration of Analyte Element in Spike (µg/unit of sample)

Slope = \frac{Absorbance}{Concentration}

FIGURE 1
**TABLE 7**

Minimum Detection Limits for Atomic Absorption Spectroscopic Analyses by Sample Type, by Element and by Atomization Method

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Element</th>
<th>Atomization Method</th>
<th>Minimum Detection Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Cd</td>
<td>CRA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.003 μg/g</td>
</tr>
<tr>
<td></td>
<td>Cd</td>
<td>Flame&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.18 μg/g</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>Flame</td>
<td>2.5 μg/g</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>Flame</td>
<td>0.2 μg/g</td>
</tr>
<tr>
<td>Kidney</td>
<td>Cd</td>
<td>CRA</td>
<td>0.01 μg/g</td>
</tr>
<tr>
<td></td>
<td>Cd</td>
<td>Flame</td>
<td>1.0 μg/g</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>Flame</td>
<td>1.3 μg/g</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>CRA</td>
<td>0.05 μg/g</td>
</tr>
<tr>
<td>Muscle</td>
<td>Cd</td>
<td>CRA</td>
<td>0.002 μg/g</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>Flame</td>
<td>1.7 μg/g</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>Flame</td>
<td>0.06 μg/g</td>
</tr>
<tr>
<td>Hair</td>
<td>Cd</td>
<td>CRA</td>
<td>0.01 μg/g</td>
</tr>
<tr>
<td></td>
<td>Cd</td>
<td>Flame</td>
<td>0.18 μg/g</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>Flame</td>
<td>0.4 μg/g</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>Flame</td>
<td>0.29 μg/g</td>
</tr>
<tr>
<td>Blood</td>
<td>Cd</td>
<td>CRA</td>
<td>0.009 μg/100 ml</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>Flame</td>
<td>2.6 μg/100 ml</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>Flame</td>
<td>2.3 μg/100 ml</td>
</tr>
<tr>
<td>Feces and</td>
<td>Cd</td>
<td>CRA</td>
<td>0.07 μg/g</td>
</tr>
<tr>
<td>Ration</td>
<td>Cd</td>
<td>Flame</td>
<td>5.7 μg/g</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>Flame</td>
<td>19 μg/g</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>Flame</td>
<td>0.7 μg/g</td>
</tr>
</tbody>
</table>

<sup>a</sup> Carbon furnace (rod) atomization

<sup>b</sup> Air-acetylene flame atomization
Liver

A one gram sample size was used for all liver analyses. Samples were digested for 50 hrs in 10 ml of concentrated HNO₃ with 2 ml of H₂O₂ added in aliquots of 1 ml at approximately 18 hr and 34 hr. The samples were evaporated to a volume of approximately 2 ml then transferred to 10 ml volumetric flasks and brought up to volume.

The 100% spike for the standard additions series contained 0.05 µg Cd, 5 µg Cu and 50 µg Zn for liver of baseline group calves and contained 5 µg Cd, 5 µg Cu and 50 µg Zn for liver of groups 1, 2, and 3 calves.

Flame atomization was used for all liver zinc and copper analyses and for cadmium analyses of groups 1, 2, and 3 liver. Baseline group liver cadmium analyses were done by carbon furnace atomization.

Kidney

Kidney cortex samples weighing 0.5 g were digested for 40-48 hrs in 3 ml of concentrated HNO₃. At 25 hrs, 0.5 ml of H₂O₂ was added. The samples were not evaporated after digestion and were transferred to 25 ml volumetric flasks and brought up to volume.

Baseline groups samples' standard additions series were spiked at the 100% level with 0.25 µg Cd, 2.5 µg Cu and 20 µg Zn. The 100% standard additions spike for groups 1, 2, and 3 samples was 2 µg Cd, 2.5 µg Cu and 100 µg Zn.

All zinc analyses and cadmium analyses of groups 1, 2, and 3 kidney samples were done by flame atomization. Carbon furnace atomization was used for cadmium in baseline group samples and for copper in all samples.
**Muscle**

Zinc analyses were done on 3 g samples of muscle digested for 108 hrs in 13 ml HNO₃ and 1 ml H₂O₂ (added at 36 hrs). Samples were evaporated to approximately 2 ml, transferred to 10 ml volumetric flasks and brought up to volume. The 100% spike for the standard additions series contained 150 μg Zn. Flame atomization was used for zinc analyses.

Cadmium and copper analyses were done on 1.5 g samples digested 84-105 hrs in 15 ml HNO₃ and 1 ml H₂O₂ (added at 69-90 hrs). Samples were evaporated to approximately 1 ml before being transferred to 10 ml volumetric flasks and made up to volume.

The standard additions series 100% spike was 0.025 μg Cd and 2.5 μg Cu. Cadmium analyses were done by carbon furnace atomization and copper by flame atomization.

**Hair**

Hair samples collected at the time calves were received from farms were prepared for analysis by digesting 0.5 g samples in 3 ml of HNO₃ and 0.5 ml of H₂O₂ for approximately 60 hrs. Digested samples were not evaporated and were made up to 10 ml exact volume.

The standard additions series 100% spike was 0.05 μg Cd, 3.5 μg Cu and 75 μg Zn. Cadmium analyses were performed with carbon furnace atomization and flame atomization was used for copper and zinc.

A sample size of 0.25 g was used for hair samples collected at the end of the 60 day experimental feeding period. Samples were heated for
34-58 hrs in 10 ml of HNO₃ and 1 ml of H₂O₂ (added at approximately 8 hrs) and then evaporated to approximately 2 ml and brought up to 10 ml exact volume.

The 100% spike in the standard additions series was 0.5 µg Cd, 2 µg Cu and 50 µg Zn. Cadmium, copper and zinc analyses were done with flame atomization.

**Blood**

Digestion was done in 3 stages for pre-feeding (0 day), 0.5 day, 1.5 day and 3.5 day blood samples. A 5 ml portion of blood was heated for 24 hrs with 10 ml of HNO₃ then evaporated to near dryness.

A second 5 ml portion of blood and another 10 ml of HNO₃ was added and heated for 24 hrs and then evaporated to near dryness. A final 10 ml of HNO₃ was added to the blood digestate and heating was resumed.

After 24 hrs, 2 ml of H₂O₂ was added and heating was continued an additional 24 hrs. Samples were evaporated to near dryness and made up to 10 ml exact volume. The standard additions series 100% spike contained 0.01 µg Cd, 10 µg Cu and 25 µg Zn.

The 8 day, 15 day, 30 day, 45 day and 60 day blood samples were digested in a single stage. Two milliliter blood samples were heated in 10 ml of HNO₃. Hydrogen peroxide (2 ml) was added at 12-24 hrs and heating was continued for a total of 37-55 hrs.

Samples were evaporated to approximately 1 ml then brought up to 10 ml exact volume. The 100% spike for the standard additions series was 0.01 µg Cd, 2 µg Cu and 5 µg Zn.
All blood cadmium analyses were done with carbon furnace atomization and all blood copper and zinc analyses were done with flame atomization.

**Feces**

A sample size of 0.2 g was used for pre-feeding (0 day) and 0.5 day feces. Samples were heated with 5 ml HNO₃ and 0.75 ml H₂O₂ (added at 27 hrs) for 53 hrs. Evaporation was not required and the samples were made up to 100 ml exact volume.

The 100% standard additions spike was 0.01 ug Cd, 4 ug Cu and 40 ug Zn. Cadmium analyses were done with carbon furnace atomization and copper and zinc analyses were done with flame atomization.

Feces collected at 1.5 days through 60 days were analyzed using a 0.5 g sample heated in 10 ml of HNO₃ for 48-88 hrs. At 24-48 hrs of heating time, 2 ml of H₂O₂ was added in 1 ml portions.

Samples were not evaporated and were made up to 50 ml exact volume. The 100% standard additions series spike was 50 ug Cd, 10 ug Cu and 100 ug Zn. All analyses were done with flame atomization.

**Ration**

Analyses of rations were done with 0.5 g samples heated for 71 hrs in 10 ml of HNO₃. At 20 hrs heating time, 1 ml of H₂O₂ was added. Samples were not evaporated and were made up to 50 ml exact volume.

The 100% standard additions spike used for the basal ration contained 0.1 ug Cd, 5 ug Cu and 20 ug Zn; and that used for the cadmium and zinc added rations contained 25 ug Cd, 5 ug Cu and 100 ug Zn. All analyzels were done with flame atomization except the cadmium
analyses of the basal ration which were done with carbon furnace atomization.

F. Statistical Analysis

Each analytical result was entered, along with coded identifying information, on a computer punch card. Data cards were read into the computer for storage. Analyte concentration data were normalized by log transformation and statistically analyzed by analysis of variance using a partial hierarchical design (Brownlee, 1965, p. 530).

The analysis of variance was carried out by a Statistical Analysis System (SAS) general linear models procedure executed through The Ohio State University, Instructional and Research Computer Center.

The partitioning of sums of squares and degrees of freedom were as follows: Sludge Exposure, 1 degree of freedom; Treatment, 2 degrees of freedom; Sludge Exposure-Treatment interaction, 2 degrees of freedom; Farm nested within Sludge Exposure, 6 degrees of freedom; Farm-Treatment nested within Sludge Exposure interaction, 12 degrees of freedom; and Duplicate Analysis Error, 24 degrees of freedom.

Two a priori tests of hypothesis were carried out in the analysis of variance. The hypothesis that no difference existed between calves exposed to sludge and calves not exposed to sludge was tested by computing the F statistic: Sludge Exposure mean square divided by Farm nested within Sludge Exposure mean square.

The hypothesis that no difference existed among treatment groups of calves was tested by computing the F statistic: Treatment mean
square divided by Farm-Treatment nested within Sludge Exposure mean square. Duncan's multiple range test for variable value was used as a post test to test for differences between means of treatment groups at \( p = 0.05 \) and at \( p = 0.01 \) levels.

Student's t test (Steel and Torrie, 1960, p. 102) was used to test for equality of male calves' and female calves' mean liver and kidney cadmium concentrations. Student's t test on paired observations (Li, 1964, p. 108) was used to test for a cadmium or cadmium and zinc feeding effect on the hair cadmium and zinc concentrations of calves.
IV. RESULTS

A. Calf Performance

Calves maintained a generally thrifty condition throughout the 60 day experimental feeding period and gained an average of approximately 10% of their body weights (Table 8). No apparent signs of cadmium or zinc toxicity were observed.

Calves, on average, consumed approximately 98.7% of the rations allowed them. Of 24 calves, 15 consumed 100% of their allotted rations while the remaining 9 consumed between 91.2% and 99.3% (Table 6).

Three calves consumed less than 95% of allotted ration. Calf no. 45 (treatment group 1) exhibited an unexplained prolonged (12 days) period of partial anorexia which contributed to his consuming only approximately 94.3% of his total allotted ration. This calf showed only occasional diarrhea during the anorectic period and did not appear to lose weight.

A chronic bloat condition resulted in the need to supplement calf no. 55 (treatment group 2) with 0.5 lb of hay per day as a preventive measure. This calf had a poor appetite, especially following his frequent episodes of bloat and consequently consumed only approximately 91.2% of his allotted ration. The bloat condition did not cause the calf to appear unthrifty, however.
TABLE 8

Body Weights of Calves at Start and at End of 60 Day Experimental Feeding Period by Treatment Group

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>No. of Calves</th>
<th>Mean Body Weight, kg</th>
<th>Increase During Feeding Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>8</td>
<td>195</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>198</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>175</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>168</td>
<td>16</td>
</tr>
</tbody>
</table>

The baseline group was not fed added cadmium or zinc and was weighed, slaughtered and sampled before the start of the 60 day feeding period. Group 1, 50 µg/g cadmium; group 2, 50 µg/g cadmium plus 200 µg/g zinc; and group 3, 50 µg/g cadmium plus 600 µg/g zinc added to ration for 60 days.
Calf no. 37 (treatment group 3) was one of the calves which had pneumonia. He recovered within 3 days. He also developed a digestive upset which lasted 7 days and was characterized by initial constipation and almost total anorexia followed by diarrhea as his appetite improved. He consumed only approximately 93% of his allotted ration.

During the 60 day experimental feeding period, 11 of the 24 calves developed pneumonia. Five calves in group 1, 4 calves in group 2 and 2 calves in group 3 were affected. Signs of pneumonia in these calves were sudden onset (usually correlated with malfunction of the ventilation system), temperature often over 106°F, depression, cough and anorexia.

They were treated with intramuscular injections of spectinomycin and recovered within 3 to 8 days without complication. Very minor or no residual lung lesions were evident at slaughter inspection.

B. Sludge Exposure Effects

Concentrations of cadmium, zinc and copper in liver, kidney cortex, muscle and hair collected from sludge exposed and non-sludge exposed calves at the time they were received from farms of origin are given in Table 9.

The analysis of variance tests for differences between means of sludge exposed and non-sludge exposed calves for each element in each sample type revealed no significant (p < 0.05) differences.

The results for each of the 3 elements in each sample type from each sampling period for treatment groups 1, 2 and 3 calves were also tested for sludge exposure effects by analysis of variance. Of 66
### TABLE 9

Cadmium (Cd), Zinc (Zn) and Copper (Cu) Concentrations in Liver, Kidney Cortex, Muscle and Hair of Sludge Exposed and Non-Sludge Exposed (Control) Calves Sampled at the Time Received from Farms

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Element</th>
<th>Sludge Exposed</th>
<th>Non-Sludge Exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liver</strong></td>
<td>Cd</td>
<td>0.061 ± 0.008</td>
<td>0.054 ± 0.009</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>49.3 ± 9.0</td>
<td>51.0 ± 6.1</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>5.0 ± 2.6</td>
<td>25.0 ± 10.4</td>
</tr>
<tr>
<td><strong>Kidney Cortex</strong></td>
<td>Cd</td>
<td>0.379 ± 0.105</td>
<td>0.278 ± 0.090</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>25.2 ± 1.3</td>
<td>23.8 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>2.2 ± 0.2</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td><strong>Muscle</strong></td>
<td>Cd</td>
<td>0.003 ± 0.001</td>
<td>0.007 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>53.1 ± 3.8</td>
<td>57.4 ± 7.8</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>1.2 ± 0.1</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td><strong>Hair</strong></td>
<td>Cd</td>
<td>0.121 ± 0.036</td>
<td>0.113 ± 0.035</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>158 ± 10</td>
<td>130 ± 3</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>4.1 ± 0.4</td>
<td>5.4 ± 0.4</td>
</tr>
</tbody>
</table>

**a** Means for liver, kidney cortex and muscle are for 4 calves and means for hair are for 16 calves. None of the tissue element means of sludge exposed calves differed significantly (p < 0.05) from the means of non-sludge exposed calves.

**b** Values for liver, kidney cortex and muscle are expressed on a wet weight basis and for hair on an air-dry basis.
comparisons of mean sample concentrations between sludge exposed and non-sludge exposed calves, only 2 showed significant (p < 0.05) differences. Non-sludge calves had higher 60 day hair cadmium concentrations and 30 day fecal copper concentrations than sludge exposed calves at the 0.05 level of statistical significance but not at the 0.01 level.

C. Liver

Concentrations of cadmium, zinc and copper in liver are presented in Table 10. Means of baseline group calves were not included in statistical tests done on means of the cadmium fed groups of calves.

The mean liver cadmium concentration of cadmium fed low level zinc calves (8.77 μg/g) was 146 times higher than that (0.06 μg/g) of the baseline group calves. The ratio of ration to liver cadmium concentrations for cadmium fed low level zinc calves was 6.4 (mean cadmium concentration of batches 1 and 2 of treatment ration 1, 56.5 μg/g (Table 5), divided by 8.77 μg/g).

Liver cadmium concentrations of low and medium level zinc calves were not significantly (p < 0.05) different from each other but were both significantly (p < 0.01) higher than liver cadmium concentrations of high level zinc calves. The mean liver cadmium concentration of high level zinc calves (4.20 μg/g) was less than one-half that (8.77 μg/g) of low level zinc calves. The liver cadmium concentration was lowered in a dose response pattern with increasing level of zinc fed.
### TABLE 10

Cadmium (Cd), Zinc (Zn) and Copper (Cu) Concentrations in Liver of Baseline Calves and of Calves Fed 50 μg/g Cadmium and 3 Levels of Zinc for 60 Days

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Cd</th>
<th>Zn</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.06 ± 0.01</td>
<td>50.2 ± 5.0</td>
<td>15.0 ± 6.2</td>
</tr>
<tr>
<td>1</td>
<td>8.77 ± 0.42&lt;sup&gt;A&lt;/sup&gt;</td>
<td>41.5 ± 2.3&lt;sup&gt;A&lt;/sup&gt;</td>
<td>7.7 ± 2.4</td>
</tr>
<tr>
<td>2</td>
<td>6.76 ± 1.02&lt;sup&gt;B&lt;/sup&gt;</td>
<td>50.3 ± 4.0&lt;sup&gt;B&lt;/sup&gt;</td>
<td>5.7 ± 1.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>4.20 ± 0.93&lt;sup&gt;A,B&lt;/sup&gt;</td>
<td>163.9 ± 21.9&lt;sup&gt;A,B&lt;/sup&gt;</td>
<td>16.2 ± 7.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Baseline group means were not included in tests of significance. Means and standard errors within a column followed by the same superscript letter are significantly different statistically; if letters are lower case (e.g., a, b, c), significance is p < 0.05 and if upper case (e.g., A, B, C), significance is p < 0.01.

<sup>b</sup> Each group contains 8 calves. The baseline group was not fed added cadmium or zinc and was sampled before the start of the 60 day feeding period. Group 1, 50 μg/g cadmium; group 2, 50 μg/g cadmium plus 200 μg/g zinc; and group 3, 50 μg/g cadmium plus 600 μg/g zinc added to ration for 60 days.
The liver zinc concentration of baseline calves was similar to that of the low and medium level zinc calves, neither of which was significantly ($p < 0.05$) different from the other. High level zinc group calves had a mean liver zinc concentration (163.9 µg/g) over 3 times higher (significant at $p < 0.01$) than calves of the other groups.

Mean liver copper concentrations were less than one-half as much in low and medium level zinc groups of calves as they were in baseline and high level zinc groups; and, the difference between medium and high level zinc calves was significant ($p < 0.05$). However, at the 1% level of statistical significance, there were no differences in liver copper concentrations among the 3 zinc levels of cadmium fed calves. The standard error was large for liver copper means, especially for the baseline and high level zinc groups of calves.

A dose-response effect was not clearly demonstrated between liver copper concentration and level of zinc fed. The medium level zinc group had the lowest mean liver copper concentration (5.7 µg/g) while the low level zinc group had an intermediate concentration (7.7 µg/g) which was well below that (16.2 µg/g) of the high level zinc group.

D. Kidney Cortex

Cadmium, zinc and copper concentrations in kidney cortex are given in Table 11. Baseline group means were not tested statistically for differences against groups 1, 2 and 3 means.

An inverse dose response effect was demonstrated between kidney cortex cadmium concentration and level of zinc feeding. Low and medium level zinc groups' kidney cortex cadmium concentrations were not
### TABLE 11

**Cadmium (Cd), Zinc (Zn) and Copper (Cu) Concentrations in Kidney Cortex of Baseline Calves and of Calves Fed 50 µg/g Cadmium and 3 Levels of Zinc for 60 Days**

**Mean ± Standard Error*, µg/g Wet Weight**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Cd</th>
<th>Zn</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.3 ± 0.1</td>
<td>24.5 ± 0.8</td>
<td>2.14 ± 0.1</td>
</tr>
<tr>
<td>1</td>
<td>36.4 ± 4.5 A</td>
<td>36.0 ± 2.4 A</td>
<td>2.30 ± 0.1 A</td>
</tr>
<tr>
<td>2</td>
<td>26.3 ± 2.5 B</td>
<td>39.6 ± 2.1 B</td>
<td>2.18 ± 0.1 A</td>
</tr>
<tr>
<td>3</td>
<td>15.5 ± 3.8 A,B</td>
<td>140.8 ± 23.1 A,B</td>
<td>2.66 ± 0.2 A,a</td>
</tr>
</tbody>
</table>

*a Baseline group means were not included in tests of significance. Means and standard errors within a column followed by the same superscript letter are significantly different statistically; if letters are lower case (e.g. a,b,c), significance is p < 0.05 and if upper case (e.g. A,B,C), significance is p < 0.01.

*b Each group contains 8 calves. The baseline group was not fed added cadmium or zinc and was sampled before the start of the 60 day feeding period. Group 1, 50 µg/g cadmium; group 2, 50 µg/g cadmium plus 200 µg/g zinc; and group 3, 50 µg/g cadmium plus 600 µg/g zinc added to ration for 60 days.
significantly (p < 0.05) different from each other; but both were significantly (p < 0.01) higher than that of the high level zinc group.

The kidney cortex mean cadmium concentration of the cadmium fed low level zinc calves (36.4 μg/g) was 121 fold greater than that of baseline calves (0.3 μg/g) and nearly twice that (15.5 μg/g) of the cadmium fed high level zinc calves. The ratio of ration to kidney cadmium concentrations for cadmium fed low level zinc calves was 1.6 (mean cadmium concentration of batches 1 and 2 of treatment ration 1, 56.5 μg/g (Table 5), divided by 36.4 μg/g).

Kidney cortex mean zinc concentrations of cadmium fed low and medium level zinc calves did not differ significantly (p < 0.05) but were both significantly (p < 0.01) lower than that of cadmium fed high level zinc calves.

High level zinc calves had a kidney cortex mean zinc concentration approximately 3.5 times greater than either low or medium level zinc calves. Cadmium fed low and medium level zinc calves had approximately 1.5 times more kidney cortex zinc concentration than baseline calves.

Kidney cortex copper concentrations were significantly higher in cadmium fed high level zinc calves than in cadmium fed low level (p < 0.05) and medium level (p < 0.01) zinc calves. Cadmium fed low and medium level zinc calves' kidney cortex copper concentrations did not differ significantly (p < 0.05) and their concentrations were similar to the copper concentrations in the kidney cortex of baseline calves.
E. Muscle

Cadmium, zinc and copper concentrations in muscle are presented in Table 12. Means of baseline group calves were not tested statistically against means of groups 1, 2 and 3 calves.

The mean muscle cadmium concentrations of cadmium fed low, medium and high level zinc calves were significantly (p < 0.05) different and demonstrated an inverse dose response effect between level of zinc fed and muscle cadmium concentration. At the 1% level of statistical significance, however, medium level zinc calves' mean muscle cadmium concentration did not differ from mean muscle cadmium concentrations of either low or high level zinc calves.

When compared with baseline group calves, the mean muscle cadmium concentrations of cadmium fed low level and high level calves were 4 times and nearly 2 times as high, respectively. The ratio of ration to muscle cadmium concentration for cadmium fed low level zinc calves was 3,531 (mean cadmium concentration of batches 1 and 2 treatment ration 1, 56.5 µg/g (Table 5), divided by the mean muscle cadmium concentration of low level zinc calves, 0.016 µg/g (Table 12).

No significant (p < 0.05) differences in mean muscle zinc or copper concentrations were present among low, medium or high level zinc groups of calves. The mean muscle zinc and copper concentrations of baseline calves were similar to those of cadmium fed calves.

F. Hair

Hair samples taken after 60 days of cadmium feeding from areas previously clipped, which actually represented hair grown during the
Table 12

Cadmium (Cd), Zinc (Zn) and Copper (Cu) Concentrations in Muscle of Baseline Calves and of Calves Fed 50 µg/g Cadmium and 3 Levels of Zinc for 60 Days

Mean ± Standard Error, µg/g Wet Weight

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Cd</th>
<th>Zn</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.004 ± 0.001</td>
<td>55.3 ± 4.1</td>
<td>1.25 ± 0.16</td>
</tr>
<tr>
<td>1</td>
<td>0.016 ± 0.003A,a</td>
<td>52.5 ± 2.0</td>
<td>1.39 ± 0.14</td>
</tr>
<tr>
<td>2</td>
<td>0.010 ± 0.001a</td>
<td>52.6 ± 2.5</td>
<td>1.26 ± 0.12</td>
</tr>
<tr>
<td>3</td>
<td>0.007 ± 0.001A,a</td>
<td>50.9 ± 4.5</td>
<td>1.26 ± 0.11</td>
</tr>
</tbody>
</table>

a Baseline group means were not included in tests of significance. Means and standard errors within a column followed by the same superscript letter are significantly different statistically; if letters are lower case (e.g. a,b,c), significance is p < 0.05 and if upper case (e.g. A,B,C), significance is p < 0.01.

b Each group contains 8 calves. The baseline group was not fed added cadmium or zinc and was sampled before the start of the 60 day feeding period. Group 1, 50 µg/g cadmium; group 2, 50 µg/g cadmium plus 200 µg/g zinc; and group 3, 50 µg/g cadmium plus 600 µg/g zinc added to ration for 60 days.
experimental feeding period, gave lower results for concentrations of cadmium, zinc and copper than those from areas not previously clipped. Consequently, only the analytical results from 60 day regrowth (previously clipped) hair is presented and discussed. For comparison purposes, mean cadmium, zinc and copper concentrations of previously clipped and not previously clipped hair are shown in Appendix C.

Hair cadmium concentration data are given in Table 13. Hair samples collected at the time calves were received from farms showed no significant (p < 0.05) differences in mean hair cadmium concentrations among groups for calves of groups 1, 2, 3 and the baseline group.

Mean hair cadmium concentrations of 60 day cadmium fed low, medium and high level zinc calves were not significantly (p < 0.05) different. The mean hair cadmium concentrations of calves 60 days after cadmium feeding were between approximately 5 and 9 times higher (significant, p < 0.05) than they were before cadmium feeding.

The results of analyses for zinc concentration in hair are presented in Table 14. At the time they were received from farms, baseline group calves had significantly (p < 0.05) lower hair zinc concentrations than groups 2 and 3 but not group 1 calves.

Hair zinc concentrations of cadmium fed low, medium and high level zinc groups of calves were not significantly (p < 0.05) different at the end of the 60 day cadmium or cadmium plus zinc feeding period nor were they significantly (p < 0.05) different from their before feeding hair zinc concentrations.
<table>
<thead>
<tr>
<th>Treatment Group&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Before Feeding</th>
<th>End of 60 Days Feeding&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.091 ± 0.031</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.167 ± 0.083</td>
<td>0.94 ± 0.15</td>
</tr>
<tr>
<td>2</td>
<td>0.106 ± 0.031</td>
<td>0.97 ± 0.20</td>
</tr>
<tr>
<td>3</td>
<td>0.104 ± 0.038</td>
<td>0.76 ± 0.09</td>
</tr>
</tbody>
</table>

<sup>a</sup> Baseline group was not fed added cadmium or zinc and was sampled before the start of the 60 day feeding period. Group 1, 50 µg/g cadmium; group 2, 50 µg/g cadmium plus 200 µg/g zinc; and group 3, 50 µg/g cadmium plus 600 µg/g zinc added to ration for 60 days.

<sup>b</sup> End of feeding mean cadmium concentrations for each treatment group were significantly (p < 0.01) higher than before feeding means.
TABLE 14

Zinc Concentration in Hair of Calves Before and 60 Days After Feeding 50 µg/g Cadmium and 3 Levels of Zinc

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Before Feeding</th>
<th>End of 60 Days Feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>132 + 10A,a</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>141 + 11</td>
<td>135 + 6</td>
</tr>
<tr>
<td>2</td>
<td>155 + 6A</td>
<td>145 + 17</td>
</tr>
<tr>
<td>3</td>
<td>147 + 8a</td>
<td>151 + 5</td>
</tr>
</tbody>
</table>

a Means within a column or a row followed by the same superscript letter are significantly different statistically; if letters are lower case (e.g. a,b,c), significance is p < 0.05 and if upper case (e.g. A,B,C) significance is p < 0.01.

b Baseline group was not fed added cadmium or zinc and was sampled before the start of the 60 day feeding period. Group 1, 50 µg/g cadmium; group 2, 50 µg/g cadmium plus 200 µg/g zinc; and group 3, 50 µg/g cadmium plus 600 µg/g zinc added to ration for 60 days.
Table 15 gives analytical results for hair copper concentrations. Hair copper concentrations of the baseline group calves and groups 1, 2 and 3 calves were not significantly (p < 0.05) different in samples collected at the time calves were received from farms (before feeding). No significant (p < 0.05) differences were observed among mean hair copper concentrations of low, medium and high level zinc groups of calves fed cadmium or cadmium plus zinc for 60 days.

G. Blood

Table 16 presents the mean blood cadmium concentrations of cadmium or cadmium plus zinc fed low, medium and high level zinc calves from before (0 days) through the end of the 60 day cadmium or cadmium plus zinc feeding period. These data are plotted in Figure 2.

Mean blood cadmium concentrations showed erratic increases starting at 0.5 days (medium level zinc calves) and 1.5 days (low and high level zinc calves) which continued through 15 days. From 30 days through 60 days, blood cadmium concentrations rose slowly but consistently in all treatment groups.

At 8 days, the mean blood cadmium concentration of the low level zinc calves was significantly (p < 0.05) higher than the mean blood cadmium concentration of the medium level zinc calves; and at 15 days it was significantly (p < 0.05) higher than the mean blood cadmium concentration of the high level zinc calves.

Low level zinc calves' mean blood cadmium concentration remained significantly higher than the mean blood cadmium concentration of medium and high level zinc calves at 30 days and 45 days (p < 0.01) and
## TABLE 15

Copper Concentration in Hair of Calves Before and 60 Days After Feeding 50 µg/g Cadmium and 3 Levels of Zinc

<table>
<thead>
<tr>
<th>Treatment Group&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Before Feeding</th>
<th>End of 60 Days Feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>4.77 ± 0.59</td>
<td>7.72 ± 0.82</td>
</tr>
<tr>
<td>1</td>
<td>4.95 ± 0.77</td>
<td>7.19 ± 0.89</td>
</tr>
<tr>
<td>2</td>
<td>4.45 ± 0.52</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4.85 ± 0.60</td>
<td>9.18 ± 1.75</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significant (p < 0.05) differences were not observed among any of the 4 treatment groups' means in the before feeding samples nor among any of the 3 treatment groups' means of samples taken at the end of 60 days feeding. No statistical tests were made to compare each treatment group's before and end of feeding means.

<sup>b</sup> Baseline group was not fed added cadmium or zinc and was sampled before the start of the 60 day feeding period. Group 1, 50 µg/g cadmium; group 2, 50 µg/g cadmium plus 200 µg/g zinc; and group 3, 50 µg/g cadmium plus 600 µg/g zinc added to ration for 60 days.
<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>0 Days</th>
<th>0.5 Days</th>
<th>1.5 Days</th>
<th>3.5 Days</th>
<th>8 Days</th>
<th>15 Days</th>
<th>30 Days</th>
<th>45 Days</th>
<th>60 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.059</td>
<td>0.036</td>
<td>0.132</td>
<td>0.081</td>
<td>0.197</td>
<td>0.223</td>
<td>0.359</td>
<td>0.389</td>
<td>0.456</td>
</tr>
<tr>
<td></td>
<td>± 0.028</td>
<td>± 0.009</td>
<td>± 0.045</td>
<td>± 0.045</td>
<td>± 0.035</td>
<td>± 0.049</td>
<td>± 0.048</td>
<td>± 0.040</td>
<td>± 0.043</td>
</tr>
<tr>
<td>2</td>
<td>0.029</td>
<td>0.136</td>
<td>0.084</td>
<td>0.077</td>
<td>0.089</td>
<td>0.139</td>
<td>0.156</td>
<td>0.186</td>
<td>0.204</td>
</tr>
<tr>
<td></td>
<td>± 0.005</td>
<td>± 0.062</td>
<td>± 0.016</td>
<td>± 0.016</td>
<td>± 0.013</td>
<td>± 0.028</td>
<td>± 0.030</td>
<td>± 0.020</td>
<td>± 0.028</td>
</tr>
<tr>
<td>3</td>
<td>0.042</td>
<td>0.039</td>
<td>0.174</td>
<td>0.066</td>
<td>0.151</td>
<td>0.086</td>
<td>0.163</td>
<td>0.206</td>
<td>0.231</td>
</tr>
<tr>
<td></td>
<td>± 0.011</td>
<td>± 0.010</td>
<td>± 0.083</td>
<td>± 0.016</td>
<td>± 0.050</td>
<td>± 0.028</td>
<td>± 0.056</td>
<td>± 0.038</td>
<td>± 0.052</td>
</tr>
</tbody>
</table>

Each mean represents 6 calves (0.5 days) or 8 calves (0 days and 1.5-60 days). Means within a column followed by the same superscript letter are significantly different statistically; if letters are lower case (e.g. a,b,c), significance is $p < 0.05$ and if upper case (e.g. A,B,C), significance is $p < 0.01$.

Group 1, 50 mg/g cadmium; group 2, 50 mg/g cadmium plus 200 mg/g zinc; and group 3, 50 mg/g cadmium plus 600 mg/g zinc added to ration for 60 days.
FIGURE 2

Cadmium concentration (mean ± standard error) in blood of calves before the start of and at intervals after feeding 50 µg/g cadmium and 3 levels of zinc. ○ = 50 µg/g cadmium with low (basal) level zinc, □ = 50 µg/g cadmium with medium (200 µg/g) level zinc, △ = 50 µg/g cadmium with high (600 µg/g) level zinc. Each mean represents 6 calves (0.5 days) or 8 calves (0 days and 1.5-60 days).
Blood Cadmium (µg/100mL)

Time after Starting to Feed Added Cadmium or Cadmium and Zinc Ration

FIGURE 2
at 60 days (p < 0.05). The mean blood cadmium concentration of low level zinc calves was nearly twice that of medium and high level zinc calves at 30, 45 and 60 days. The mean 60 day blood cadmium concentration of cadmium fed low level zinc calves, 0.456 μg/100 ml, was 10.6 times higher than 0.043 μg/100 ml which was the mean blood cadmium concentration of treatment groups 1, 2 and 3 calves combined at 0 days (pre-feeding).

Table 17 gives mean blood zinc concentrations of cadmium or cadmium plus zinc fed calves from 0 days through 60 days. Blood zinc data are plotted in Figure 3. Over the 60 day feeding period, mean blood zinc concentrations declined slowly in the low and medium level zinc calves and increased by approximately 1.3 times in the high level zinc calves.

Mean blood zinc concentrations of all treatment groups were not significantly (p < 0.05) different at 0 days and 0.5 days. At 1.5 days, the mean blood zinc concentration of high level zinc calves became significantly (p < 0.05) greater than the mean blood concentration of low level zinc calves; and from 3.5 days through 60 days it remained significantly (p < 0.01) higher than the mean blood zinc concentrations of both low and medium level zinc calves.

No significant (p < 0.05) differences were observed at any sampling time between the mean blood zinc concentrations of low and medium level zinc calves.

Table 18 gives mean blood copper concentrations of cadmium or cadmium plus zinc fed calves from 0 days through 60 days. These blood
Table 17

Zinc Concentration in Blood of Calves before the Start of and at Intervals after Feeding 50 μg/g Cadmium and 3 Levels of Zinc

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>0 Days</th>
<th>0.5 Days</th>
<th>1.5 Days</th>
<th>3.5 Days</th>
<th>8 Days</th>
<th>15 Days</th>
<th>30 Days</th>
<th>45 Days</th>
<th>60 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>310 ± 22</td>
<td>316 ± 24</td>
<td>303 ± 18</td>
<td>278 ± 18</td>
<td>266 ± 18</td>
<td>280 ± 18</td>
<td>271 ± 18</td>
<td>265 ± 18</td>
<td>273 ± 18</td>
</tr>
<tr>
<td>2</td>
<td>307 ± 8</td>
<td>298 ± 12</td>
<td>317 ± 10</td>
<td>297 ± 10</td>
<td>277 ± 10</td>
<td>300 ± 10</td>
<td>286 ± 10</td>
<td>289 ± 10</td>
<td>281 ± 10</td>
</tr>
<tr>
<td>3</td>
<td>334 ± 12</td>
<td>338 ± 13</td>
<td>366 ± 15</td>
<td>373 ± 12</td>
<td>365 ± 12</td>
<td>407 ± 18</td>
<td>426 ± 18</td>
<td>431 ± 19</td>
<td>426 ± 17</td>
</tr>
</tbody>
</table>

Each mean represents 6 calves (0.5 days) or 8 calves (0 days and 1.5-60 days). Means within a column followed by the same superscript letter are significantly different statistically; if letters are lower case (e.g., a,b,c), significance is p < 0.05 and if upper case (e.g., A,B,C), significance is p < 0.01.

a Group 1, 50 μg/g cadmium; group 2, 50 μg/g cadmium plus 200 μg/g zinc; and group 3, 50 μg/g cadmium plus 600 μg/g zinc added to ration for 60 days.
FIGURE 3
Zinc concentration (mean ± standard error) in blood of calves before the start of and at intervals after feeding 50 µg/g cadmium and 3 levels of zinc. ◇ = 50 µg/g cadmium with low (basal) level zinc, □ = 50 µg/g cadmium with medium (200 µg/g) level zinc, △ = 50 µg/g cadmium with high (600 µg/g) level zinc. Each mean represents 6 calves (0.5 days) or 8 calves (0 days and 1.5-60 days).
Blood Zinc (µg/100ml)

Time after Starting to Feed Added Cadmium or Cadmium and Zinc Rations

FIGURE 3
# TABLE 18
Copper Concentration in Blood of Calves before the Start of and at Intervals after Feeding 50 µg/g Cadmium and 3 Levels of Zinc

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>0 Days</th>
<th>0.5 Days</th>
<th>1.5 Days</th>
<th>3.5 Days</th>
<th>8 Days</th>
<th>15 Days</th>
<th>30 Days</th>
<th>45 Days</th>
<th>60 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>68</td>
<td>61</td>
<td>65</td>
<td>75</td>
<td>78</td>
<td>73</td>
<td>67</td>
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<td></td>
<td>± 5</td>
<td>± 8</td>
<td>± 6</td>
<td>± 4</td>
<td>± 8</td>
<td>± 8</td>
<td>± 8</td>
<td>± 8</td>
<td>± 8</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>59</td>
<td>65</td>
<td>74</td>
<td>76</td>
<td>68</td>
<td>58</td>
<td>53</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>± 6</td>
<td>± 8</td>
<td>± 7</td>
<td>± 9</td>
<td>± 8</td>
<td>± 7</td>
<td>± 7</td>
<td>± 6</td>
<td>± 7</td>
</tr>
<tr>
<td>3</td>
<td>73</td>
<td>69</td>
<td>71</td>
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<td>73</td>
<td>69</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>± 6</td>
<td>± 8</td>
<td>± 6</td>
<td>± 7</td>
<td>± 7</td>
<td>± 6</td>
<td>± 6</td>
<td>± 5</td>
<td></td>
</tr>
</tbody>
</table>

*Each mean represents 6 calves (0.5 days) or 8 calves (0 days and 1.5-60 days). Means within a column followed by the same superscript letter are significantly different statistically; if letters are lower case (e.g. a,b,c), significance is p < 0.05 and if upper case (e.g. A,B,C), significance is p < 0.01.

Group 1, 50 µg/g cadmium; group 2, 50 µg/g cadmium plus 200 µg/g zinc; and group 3, 50 µg/g cadmium plus 600 µg/g zinc added to ration for 60 days.
copper data are plotted in Figure 4. No significant ($p < 0.05$) differences were observed in the mean blood copper concentrations of low, medium or high level zinc calves except at 30 days and 45 days when the mean blood copper concentrations of medium level zinc calves were lower (significant at $p < 0.05$ but not at $p < 0.01$) than those of high level zinc calves.

H. Feces

Mean fecal cadmium concentrations of cadmium or cadmium plus zinc fed calves from 0 days through 60 days are tabulated in Table 19 and are plotted in Figure 5. Low, medium and high level zinc calves' mean fecal cadmium concentrations did not differ significantly ($p < 0.05$) within any sampling time with the exception of 0 days and 3.5 days.

The mean fecal cadmium concentration of low level zinc calves was higher (significant at $p < 0.05$ but not at $p < 0.01$) than that of medium and high level zinc calves at 0 days. At 3.5 days, medium level zinc calves' mean fecal cadmium concentration was higher (significant at $p < 0.05$ but not at $p < 0.01$) than that of high level zinc calves.

The cadmium content of feces of calves fed 50 μg/g cadmium increased from an average pre-feeding level of 1.2 μg/g at 0 days to 6.8 μg/g at 0.5 days, 126 μg/g at 1.5 days and leveled off at approximately 160 μg/g from 3.5 through 60 days.

Table 20 gives the mean fecal zinc concentration of cadmium or cadmium plus zinc fed calves from 0 days through 60 days. These data are plotted in Figure 6. Mean fecal zinc concentrations were not
Cooper concentration (mean ± standard error) in blood of calves before the start of and at intervals after feeding 50 μg/g cadmium and 3 levels of zinc. ○ = 50 μg/g cadmium with low (basal) level zinc, □ = 50 μg/g cadmium with medium (200 μg/g) level zinc, △ = 50 μg/g cadmium with high (600 μg/g) level zinc. Each mean represents 6 calves (0.5 days) or 8 calves (0 days and 1.5-60 days).
Figure 4

Blood Copper (μg/100ml) vs. Time after Starting to Feed Added Cadmium or Cadmium and Zinc Rations.
TABLE 19
Cadmium Concentration in Feces of Calves before the Start of and at Intervals after Feeding 50 µg/g Cadmium and 3 Levels of Zinc

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>0 Days</th>
<th>0.5 Days</th>
<th>1.5 Days</th>
<th>3.5 Days</th>
<th>8 Days</th>
<th>15 Days</th>
<th>30 Days</th>
<th>45 Days</th>
<th>60 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.62±0.27</td>
<td>6.50</td>
<td>122</td>
<td>158</td>
<td>155</td>
<td>156</td>
<td>155</td>
<td>163</td>
<td>169</td>
</tr>
<tr>
<td>2</td>
<td>1.06±0.21</td>
<td>7.60</td>
<td>127</td>
<td>166±3</td>
<td>161</td>
<td>151</td>
<td>160</td>
<td>164</td>
<td>162</td>
</tr>
<tr>
<td>3</td>
<td>1.03±0.22</td>
<td>6.55</td>
<td>129</td>
<td>153±4</td>
<td>165</td>
<td>161</td>
<td>155</td>
<td>160</td>
<td>166</td>
</tr>
</tbody>
</table>

*Each mean represents 8 calves. Means within a column followed by the same superscript letter are significantly different statistically; if letters are lower case (e.g. a, b, c), significance is p < 0.05 and if upper case (e.g. A, B, C), significance is p < 0.01.

*Group 1, 50 µg/g cadmium; group 2, 50 µg/g cadmium plus 200 µg/g zinc; and group 3, 50 µg/g cadmium plus 600 µg/g zinc added to ration for 60 days.
FIGURE 5

Cadmium concentration (mean ± standard error) in feces of calves before the start of and at intervals after feeding 50 µg/g cadmium and 3 levels of zinc. ○ = 50 µg/g cadmium with low (basal) level zinc, □ = 50 µg/g cadmium with medium (200 µg/g) level zinc, △ = 50 µg/g cadmium with high (600 µg/g) level zinc. Each mean represents 8 calves.
Fecal Cadmium (µg/g)

Days

Time after Starting to Feed Added Cadmium or Cadmium and Zinc Rations

FIGURE 5
### TABLE 20

Zinc Concentration in Feces of Calves before the Start of and at Intervals after Feeding 50 μg/g Cadmium and 3 Levels of Zinc

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>0 Days</th>
<th>0.5 Days</th>
<th>1.5 Days</th>
<th>3.5 Days</th>
<th>8 Days</th>
<th>15 Days</th>
<th>30 Days</th>
<th>45 Days</th>
<th>60 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>191</td>
<td>204&lt;sup&gt;a&lt;/sup&gt;</td>
<td>162&lt;sup&gt;A&lt;/sup&gt;</td>
<td>148&lt;sup&gt;A&lt;/sup&gt;</td>
<td>172&lt;sup&gt;A&lt;/sup&gt;</td>
<td>141&lt;sup&gt;A&lt;/sup&gt;</td>
<td>147&lt;sup&gt;A&lt;/sup&gt;</td>
<td>151&lt;sup&gt;A&lt;/sup&gt;</td>
<td>159&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>± 13</td>
<td>± 13</td>
<td>± 9</td>
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<td>± 6</td>
</tr>
<tr>
<td>2</td>
<td>191</td>
<td>295</td>
<td>464&lt;sup&gt;A&lt;/sup&gt;</td>
<td>598&lt;sup&gt;A&lt;/sup&gt;</td>
<td>597&lt;sup&gt;A&lt;/sup&gt;</td>
<td>552&lt;sup&gt;A&lt;/sup&gt;</td>
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<td>± 37</td>
</tr>
<tr>
<td>3</td>
<td>185</td>
<td>364&lt;sup&gt;a&lt;/sup&gt;</td>
<td>968&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1,212&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1,284&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1,298&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1,338&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1,420&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1,401&lt;sup&gt;A&lt;/sup&gt;</td>
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<td>± 13</td>
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<td>± 37</td>
<td>± 48</td>
<td>± 68</td>
<td>± 22</td>
<td>± 52</td>
</tr>
</tbody>
</table>

<sup>a</sup> Each mean represents 8 calves. Means within a column followed by the same superscript letter are significantly different statistically; if letters are lower case (e.g. a,b,c), significance is p < 0.05 and if upper case (e.g. A,B,C), significance is p < 0.01.

<sup>b</sup> Group 1, 50 μg/g cadmium; group 2, 50 μg/g cadmium plus 200 μg/g zinc; and group 3, 50 μg/g cadmium plus 600 μg/g zinc added to ration for 60 days.
FIGURE 6

Zinc concentration (mean ± standard error) in feces of calves before the start of and at intervals after feeding 50 μg/g cadmium and 3 levels of zinc. ○ = 50 μg/g cadmium with low (basal) level zinc, □ = 50 μg/g cadmium with medium (200 μg/g) level zinc, △ = 50 μg/g cadmium with high (600 μg/g) level zinc. Each mean represents 8 calves.
FIGURE 6

Time after Starting to Feed Added Cadmium or Cadmium and Zinc Rations
significantly (p < 0.05) different for low, medium or high level zinc calves at 0 days.

High level zinc calves had a significantly (p < 0.05) higher mean fecal zinc concentration than that of low level zinc calves at 0.5 days. At each sampling time from 1.5 through 60 days, high level zinc calves had significantly (p < 0.01) higher mean fecal zinc concentrations than those of medium level zinc calves which, in turn, had significantly (p < 0.01) higher mean fecal zinc concentrations than those of low level zinc calves.

Fecal zinc concentrations averaged 189 μg/g at 0 days. At 8 days, mean fecal zinc concentrations for low, medium and high level zinc calves were 172, 597 and 1,284 μg/g, respectively. Finally, at 60 days, mean fecal zinc concentrations were 159, 635 and 1,401 μg/g for low, medium and high level zinc calves, respectively.

The mean copper concentrations in feces of calves fed cadmium or cadmium plus zinc from 0 days through 60 days are tabulated in Table 21 and plotted in Figure 7.

Mean fecal copper concentrations of low, medium and high level zinc calves were not significantly (p < 0.05) different within any sampling time except for 15 days and 30 days. High level zinc calves had higher (significant at p < 0.05 but not at p < 0.01) mean fecal copper concentrations than those of low level zinc calves at 15 and 30 days.

Fecal copper concentrations began to decrease 1.5 days after cadmium feeding was started, reached plateau levels between 8 days and
TABLE 21

Copper Concentration in Feces of Calves before the Start of and at Intervals after Feeding 50 μg/g Cadmium and 3 Levels of Zinc

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>0 Days</th>
<th>0.5 Days</th>
<th>1.5 Days</th>
<th>3.5 Days</th>
<th>8 Days</th>
<th>15 Days</th>
<th>30 Days</th>
<th>45 Days</th>
<th>60 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26.8 + 1.0</td>
<td>28.2 + 1.3</td>
<td>20.8 + 1.4</td>
<td>18.8 + 0.8</td>
<td>18.8 + 0.9</td>
<td>17.6 + 0.9</td>
<td>17.5 + 0.7</td>
<td>20.8 + 1.9</td>
<td>21.4 ± 0.9</td>
</tr>
<tr>
<td>2</td>
<td>27.1 + 0.9</td>
<td>27.8 + 1.6</td>
<td>20.3 + 1.5</td>
<td>20.0 + 1.8</td>
<td>18.4 + 0.9</td>
<td>18.3 + 0.8</td>
<td>18.3 + 1.1</td>
<td>20.6 + 1.0</td>
<td>22.4 ± 1.7</td>
</tr>
<tr>
<td>3</td>
<td>29.5 + 2.1</td>
<td>30.3 + 1.8</td>
<td>29.8 + 1.7</td>
<td>25.6 + 0.9</td>
<td>21.9 + 2.0</td>
<td>21.6 + 1.6</td>
<td>21.6 + 1.3</td>
<td>20.8 + 1.2</td>
<td>20.7 ± 1.3</td>
</tr>
</tbody>
</table>

* Each mean represents 8 calves. Means within a column followed by the same superscript letter are significantly different statistically; if letters are lower case (e.g. a,b,c), significance is p < 0.05 and if upper case (e.g. A,B,C), significance is p < 0.01.

* Group 1, 50 μg/g cadmium; group 2, 50 μg/g cadmium plus 200 μg/g zinc; and group 3, 50 μg/g cadmium plus 600 μg/g zinc added to ration for 60 days.
FIGURE 7

Copper concentration (mean ± standard error) in feces of calves before the start of and at intervals after feeding 50 μg/g cadmium and 3 levels of zinc. ○ = 50 μg/g cadmium with low (basal) level zinc, □ = 50 μg/g cadmium with medium (200 μg/g) level zinc, △ = 50 μg/g cadmium with high (600 μg/g) level zinc. Each mean represents 8 calves.
FIGURE 7

Fecal Copper (µg/g) over Time after Starting to Feed Added Cadmium or Cadmium and Zinc Ration

0 0.5 1.5 3.5 8 15 30 45 60
Days

Time after Starting to Feed Added Cadmium or Cadmium and Zinc Ration
30 days and then increased somewhat for low and medium level zinc groups between 30 days and 60 days. Mean fecal copper concentrations among all groups over all sampling times ranged between 17.5 and 30.3 \( \mu g/g \).

Table 22 gives the proportion (feces concentration divided by ration concentration) of 60 day feces concentration to ration concentration for cadmium, zinc and copper. Calves' 60 day feces contained 3.4, 2.8, 2.4, 2.1 and 3.3 times the rations' concentrations of cadmium, low level zinc, medium level zinc, high level zinc and copper, respectively.
TABLE 22

Cadmium (Cd), Zinc (Zn) and Copper (Cu) Concentrations in Rations\textsuperscript{a}
Fed to Calves and in Calves' Feces\textsuperscript{b} after 60 Days of Ration Feeding
and the Feces Concentration to Ration Concentration Proportion
by Element

<table>
<thead>
<tr>
<th>Element</th>
<th>Ration</th>
<th>Feces</th>
<th>Feces Concentration/Ration Concentration</th>
</tr>
</thead>
<tbody>
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<td>Cd</td>
<td>49</td>
<td>166</td>
<td>3.4</td>
</tr>
<tr>
<td>Zn</td>
<td>Low Level</td>
<td>56</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>Medium Level</td>
<td>260</td>
<td>635</td>
</tr>
<tr>
<td></td>
<td>High Level</td>
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<td>1,401</td>
</tr>
<tr>
<td>Cu</td>
<td>7.3</td>
<td>23.9</td>
<td>3.3</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Mean element concentration of batches (1 and 2) and treatment rations of similar nominal element content (i.e. for cadmium and copper, the mean concentration of batches 1 and 2 treatment rations 1, 2 and 3; and for zinc, the mean concentration of batches 1 and 2 for treatment ration 1 (low level), for treatment ration 2 (medium level) and for treatment ration 3 (high level).

\textsuperscript{b} Mean 60 day fecal element concentration of all treatment groups of calves being fed rations with the same nominal element concentration.
V. DISCUSSION

A. Calf Performance

Calves fed 50 µg/g cadmium only or with 200 or 600 µg/g zinc for 60 days in this experiment showed no apparent toxic effects attributable to either cadmium or zinc. The anorexia observed in a group 1 (low zinc) and in a group 3 (high zinc) calf did not share high zinc feeding as a possible common causal factor and the absence of similar signs in the remainder of the calves discounts the probability of cadmium as a cause. The chronic bloat condition of calf no. 55 began before the feeding of experimental rations started.

In other studies, reduced rate of gain has been observed in calves fed 160 µg/g cadmium (Powell et al., 1964) and in lambs fed 30 and 60 µg/g cadmium (Doyle et al., 1974). Ott et al. (1966a) reported that rates of gain in lambs fed 500 and 1,000 µg/g zinc for 10 weeks were increased over those of controls. Calves in the present study gained approximately 10% of their starting body weight over 60 days which is the equivalent of only 0.3 kg/day gain. This would be considered a low rate of gain for commercially fed cattle; however, the adjustment to weaning and ration change and the environmental stress of confinement combined with the restricted rate of feeding could easily account for this.
The signs of cadmium toxicity in calves, unthrifty appearance, rough hair coat, dehydration, dry and scaly skin, loss of hair, mouth lesions, shrunken scrotum, and sore and enlarged joints reported by Powell et al. (1964) using rations containing 640 μg/g cadmium were notably absent in the calves in this study. The calves' hair coats generally became more sleek over the 60 days of cadmium or cadmium plus zinc feeding; and the calves appeared to be alert and comfortable.

B. Sludge Exposure Effects

Calves derived from sludge receiving farms have potential exposure to increased soil and pasture levels of cadmium, zinc and copper (Chaney, 1973). Translocation of cadmium and zinc to cattle tissues and alterations of tissue copper concentrations have been reported in cattle grazing cadmium and zinc contaminated pastures (Dorn et al., 1974b; Kienholz et al., 1976).

In this study, it was necessary to utilize cattle from sludge receiving and control (non-sludge receiving) farms. Because of this possible heavy metals loading or conditioning factor, statistical (analysis of variance) comparisons of analytical results for hair and tissue samples collected at the time calves were received from farms were made between sludge exposed and non-sludge exposed calves. No significant (p < 0.05) differences were observed (Table 9).

To further ascertain whether sludge exposure might have conditioned calves to respond differently to the feeding of cadmium and cadmium plus zinc, analysis of variance comparisons between sludge
exposed and non-sludge exposed calves were made by sample type for all analytical results from cadmium and cadmium plus zinc fed calves. No significant ($p < 0.05$) differences were observed in the 66 comparisons with the exception of higher (significant at $p < 0.05$ but not at $p < 0.01$) 60 day hair cadmium and 30 day fecal copper concentrations in non-sludge exposed calves than in sludge exposed calves. Therefore, it can be concluded that sludge exposure had little or no effect on the concentrations of cadmium, zinc and copper in the test samples and that the calves from sludge exposed and non-sludge exposed farms could be combined for analysis of the feed trial data.

C. Liver

In comparison with baseline calves, feeding calves 50 $\mu$/g cadmium without supplemental zinc for 60 days caused a 146-fold increase in liver cadmium concentration. A substantial increase in liver cadmium concentration with the feeding of 50 $\mu$/g cadmium (approximately 385 times the 0.13 $\mu$/g level of cadmium in the basal ration) would be expected from the results reported from other cadmium feeding experiments in ruminants.

Doyle et al. (1974) reported liver cadmium concentrations (dry weight basis) of 62.7 and 276 $\mu$/g for lambs fed cadmium 191 days at levels of 30 and 60 $\mu$/g, respectively. Calves fed 640 and 2,560 $\mu$/g cadmium for 12 weeks had liver cadmium concentrations of 511 and 455 $\mu$/g (dry weight basis), respectively (Powell et al., 1964).

Converting the above liver cadmium data of Doyle et al. (1974) and Powell et al. (1964) to a wet weight basis (assume 80% liver moisture
content) and computing the ration cadmium concentration to liver cadmium concentration ratios reveals the following: Lambs fed 30 and 60 μg/g cadmium and calves fed 640 and 2,560 μg/g cadmium had ratio to liver cadmium ratios of 2.3, 1.1, 6.3 and 28, respectively. These compare with a ratio of 6.4 for the low level zinc group calves in this study which were fed 50 μg/g cadmium for 60 days.

Obviously, liver cadmium concentrations do not directly reflect ration cadmium concentrations on a uniform basis. The lambs of Doyle et al. (1974) fed cadmium the longest (191 days) had the highest liver cadmium in relation to ration cadmium while the extremely high level of 2,560 μg/g cadmium in the ration fed to calves for 12 weeks (Powell et al., 1964) actually resulted in lower liver cadmium than the feeding of 640 μg/g cadmium. If liver cadmium concentration is to be used to estimate ration cadmium concentration, further work needs to be done to take into account the time as well as the level of cadmium feeding. Extremely high (2,560 μg/g) levels of dietary cadmium appear to negatively influence the liver's ability to accumulate cadmium.

Although reported liver cadmium concentrations are consistently lower than those of kidney cortex in experiments, including this study which involve the administration of cadmium to animals, the liver is apparently the primary organ of cadmium uptake and metabolism (Frazier and Puglese, 1978; Cain and Skilleter, 1980; Shaikh and Smith, 1976). Cain and Skilleter (1980) found that liver parenchymal cells accumulated cadmium more readily than non-parenchymal cells and were the site of metallothionein synthesis in the liver. They proposed that
uptake and metabolism of injected CdCl₂ was restricted nearly exclusively to liver parenchymal cells.

Stonard and Webb (1976) found that cadmium accumulation reached a steady state value in the liver while kidney accumulation continued throughout an experiment in which rats were fed 100 µg/g cadmium for 40 weeks. This phenomenon would help account for the lower liver cadmium levels in comparison to those of kidney cortex observed in these calves.

The chemical form in which cadmium is presented to tissues influences its uptake. Liver preferentially takes up Cd²⁺ (possibly bound to proteins other than metallothionein) whereas kidney preferentially takes up cadmium-thionein. The intestinal mucosa apparently is capable of absorbing both forms. When mice were given equivalent amounts of ¹⁰⁹Cd either as ¹⁰⁹CdCl₂ or as ¹⁰⁹Cd-thionein by injection (Johnson and Foulkes, 1980; Nordberg, 1978) or orally (Cherian et al., 1978), the mice which received ¹⁰⁹CdCl₂ had greater uptake of ¹⁰⁹Cd in liver; whereas, those which received ¹⁰⁹Cd-thionein had greater uptake of ¹⁰⁹Cd in kidney.

Frazier and Puglese (1978) found that cadmium taken up by the liver after intravenous injection of CdCl₂ into rats was initially bound to high molecular weight macromolecules and that after 1 hr, the cadmium progressively shifted into newly synthesized metallothioneins. They reported that cadmium uptake by the liver was 90% complete within 1 hr of CdCl₂ injection; and because this was prior to induced cadmium-thionein synthesis, it constituted evidence that hepatic cadmium uptake is not dependent on metallothionein but is probably a simple diffusion process.
In this study, the salt, CdCl₂, was the form of cadmium fed to calves. The liver would be expected to be the organ of primary uptake of cadmium absorption from the gastrointestinal tract and the site of its binding to metallothionein.

Although the liver cadmium concentrations of low and medium level zinc calves were not statistically different, they varied so that the pattern which appeared when they were compared with the liver cadmium concentration of the high level zinc calves was that of an inverse dose response effect: Liver cadmium concentration decreased in response to an increase in the level of zinc fed.

Petering et al. (1979b) reports zinc effects on liver cadmium and kidney cadmium in rats similar to the findings in calves of this study. The liver and kidney cadmium concentrations in rats receiving cadmium were lower in rats receiving 40 µg/ml zinc in drinking water than in rats receiving 5 µg/ml zinc.

In rats, a sex difference in tissue cadmium accumulation has been reported. Cadmium accumulation in the liver and kidney of female rats was greater than that of male rats in experiments in which the rats were given cadmium in drinking water (El-gazzar et al., 1978; Petering et al., 1979a).

Calves available to this study were of both sexes. Through the random assignment process, groups 1 and 2 each contained 4 males and 4 females while group 3 contained 3 males and 5 females. The near equalization of sexes in the treatment groups should have substantially reduced any sex bias of the results of this study.
Although the liver cadmium concentrations and the kidney cadmium concentrations of female calves were in most cases higher, they were not significantly (p < 0.05) different from those of male calves within each treatment group. Mean liver and kidney cadmium concentrations for male and female calves in each treatment group are presented in Appendix B.

The liver zinc concentrations of baseline group calves can only be compared with those of cadmium fed calves in a very general manner since baseline calves were fed greatly different rations prior to sampling than were the cadmium fed calves. Nonetheless, liver zinc analysis results given in Table 10 indicate that the baseline, low level zinc and medium level zinc calves had essentially the same liver zinc concentrations.

The zinc concentrations of the rations of low level zinc and medium level zinc calves were approximately 56 and 260 μg/g, respectively (Table 22). The rations of the baseline calves, predominantly pasture with trace mineral supplement variably available, would be expected to contain less than 56 μg/g zinc. Considering experimental evidence which associates increases in liver zinc concentrations with cadmium treatment along with the dietary zinc history of these calves, it would be expected that low level and medium level zinc calves would have liver zinc concentrations considerably higher than those of baseline calves.

Liver zinc concentrations have been shown to increase in rats, lambs and calves when they were given substantial doses of cadmium.
Rats fed 100 µg/g cadmium (Julshamn et al., 1977; Stonard and Webb, 1976) and rats given cadmium in drinking water (Petering et al., 1979b) had elevated liver zinc levels. Doyle and Pfander (1975) reported increased liver zinc concentrations in lambs fed 30 and 60 µg/g cadmium for 191 days. Calves fed 640 and 2,560 µg/g cadmium showed increased liver zinc concentrations (Powell et al., 1964).

The relationship of cadmium and zinc in metallothionein binding is a basis upon which to explain liver zinc increases in association with cadmium loading. Cadmium injected into rats resulted in systemic mobilization of zinc followed by the synthesis of liver metallothionein which contained bound zinc on 2 of 8 possible binding sites (Winge et al., 1978).

Frazier and Puglese (1978) reported that following injection of CdCl₂ in rats, hepatic zinc was initially displaced from high molecular weight macromolecules but that within 6 hrs, hepatic zinc concentration had increased due to zinc binding on excess binding sites of newly synthesized cadmium-thionein.

The findings of Leber and Miya (1976) would offer the rationale of a net zinc displacement from metallothioneins by cadmium. They found that zinc-induced liver metallothioneins contained only zinc; whereas, those induced by cadmium contained both cadmium and zinc. Injected cadmium displaced zinc from both cadmium- and zinc-metallothioneins. Their report did not indicate whether the injected cadmium, in inducing additional cadmium-thionein, might thereby produce excess binding sites
for zinc as reported by Frazier and Puglese (1978) and by Winge et al. (1978) above.

The cadmium level fed the calves in this study, 50 μg/g, was lower than that fed to rats and calves noted above and may have been too low to produce an increase in liver zinc concentration. On the other hand, lambs fed 30 and 60 μg/g cadmium (Doyle and Pfander, 1975) did show the effect. The 60 day feeding period used in this study may have been too short to elicit the liver zinc increase; however, the high level zinc calves did demonstrate a 3 fold increase in liver zinc over 60 days.

The 3 fold elevation in liver zinc concentration of the high level zinc calves is an indication that the homeostatic control mechanism for zinc was overcome at the 600 μg/g dietary zinc level. Miller et al., (1970) noted a similar breakdown in zinc homeostasis in calves between the 200 and 600 μg/g zinc feeding level and Ott et al. (1966b) reported the phenomenon in lambs but at the much higher levels of 2,000 and 4,000 μg/g zinc feeding.

Feeding cadmium at 50 μg/g to calves in this study failed to cause an expected increase in liver zinc concentration. The effect was not seen even when zinc was fed supplementally at a level of 200 μg/g. Supplemental zinc at 600 μg/g did increase liver zinc concentration over 3 fold in calves fed 50 μg/g cadmium but the increase was more likely the result of a failure in zinc homeostatic control mechanisms rather than a result of cadmium feeding.

This study indicates that liver zinc concentration is a poor indicator of dietary zinc concentrations of normal to 200 μg/g levels
due to the homeostatic metabolic control of zinc. At dietary zinc levels between 200 to 600 µg/g and above, homeostasis begins to break down and liver zinc concentrations begin to increase in response to increased dietary zinc concentrations.

Liver copper concentrations in low and medium level zinc calves of less than one-half those of baseline and high level zinc calves (Table 10) indicate that cadmium feeding caused a depression of liver copper concentrations which was blocked by feeding the high level of zinc.

A dose response effect was not clearly shown, however. The medium level zinc calves' liver copper concentration was lowest and there was no significant difference between the liver copper concentrations of the low level zinc calves and the high level zinc calves. Large standard errors of the means of these data indicate either a lack of uniformity in the animals' responses to the treatment or nonuniform pre-existing liver copper concentrations which did not respond appreciably to treatment.

There are no reports of zinc being a factor in increasing liver copper. The opposite effect, that of zinc causing a decrease in liver copper concentration was reported for sheep (Allen and Masters, 1980; Ott et al., 1966b) and for calves (Ott et al., 1966c). Julshamn et al. (1977) and Petering et al. (1979b) reported no change in liver copper concentration in rats fed cadmium. Cadmium feeding decreased the liver copper concentrations of calves in this study and the simultaneous feeding of supplemental zinc blocked this effect.
D. Kidney Cortex

Feeding calves 50 μg/g cadmium without supplemental zinc increased kidney cadmium concentrations (Table 11) 121 fold over those of baseline calves in this study. The ration cadmium concentration to kidney cadmium concentration ratio for low level zinc calves fed 50 μg/g cadmium for 60 days was 1.6 (50 μg/g divided by 36.4 μg/g).

The kidney is an organ active in cadmium metabolism and is known to acquire cadmium both by primary uptake of cadmium absorbed from the gastrointestinal tract (Cherian et al., 1978) and by secondary accumulation of cadmium-thionein from the liver (Johnson and Foulkes, 1980) and perhaps other tissues. Cadmium-metallothionein is filtered by the kidney and reabsorbed in the renal tubules (Foulkes, 1978) where it causes necrosis of proximal renal tubular cells (Cherian et al., 1976) as it is catabolized and releases Cd^{2+} ions (Webb and Etienne, 1977).

In this study, kidney cortex cadmium concentrations (Table 11) were lowered in a dose response pattern with increasing levels of supplemental zinc feeding in calves fed 50 μg/g cadmium for 60 days. A dose response effect is not clearly shown due to the lack of significant (p < 0.05) difference between kidney cortex cadmium concentrations of low and medium level zinc calves.

Kidney zinc concentrations in calves in this study (Table 11) increased as would be expected with increasing levels of dietary zinc; and, as was noted for liver zinc concentrations, the high level zinc
calves' kidney zinc concentrations were markedly increased (3.5 fold) over those of the low and medium level zinc calves indicating a failure in zinc homeostasis. Kidney zinc concentrations would not be useful in estimating dietary zinc concentrations of less than 200 μg/g.

A relationship between kidney cadmium and kidney zinc concentrations is reported for rats, lambs and horses. Petering et al. (1979b) reported elevated kidney zinc levels in rats given cadmium in drinking water for 39 weeks; however, Julshamn et al. (1977) found no effect on kidney zinc concentrations in rats fed 100 μg/g cadmium for 9 weeks. Kidney zinc concentrations in lambs were increased by feeding 30 and 60 μg/g cadmium for 191 days (Doyle and Pfander, 1975). Nordberg et al. (1979) found high kidney cadmium concentrations associated with high kidney zinc concentrations in normal horses and noted that the excesses of both cadmium and zinc were in metallothionein.

Most of the relationships cited above for cadmium and zinc levels in both liver and kidney were based on the premise that cadmium was the variable entity and that zinc was sufficiently and passively available to respond. Dietary cadmium levels were held constant in this study while dietary zinc levels were varied in an attempt to ascertain the effects of zinc on resulting tissue cadmium concentrations. Under these conditions, both liver and kidney cadmium concentrations were negatively, rather than positively (as would be expected from published studies), correlated with liver and kidney zinc concentrations.
Two explanations could account for this unexpected relationship. First there is the possibility that zinc in higher available levels to tissues caused a net displacement of cadmium, presumably from metallothioneins, which led to a lower ultimate cadmium body burden through a higher cadmium excretion rate. The second possibility is that zinc interfered with cadmium absorption from the gastrointestinal tract so that the amount of cadmium absorbed and available for uptake was inversely related to the level of zinc fed. Additional experimental work to evaluate the effect of zinc on cadmium absorption in the gastrointestinal tract of calves would be needed to resolve the above question.

The kidney cortex copper concentration (Table 11) of calves fed 50 μg/g cadmium for 60 days was significantly (p < 0.05) increased by high (600 μg/g) level zinc dietary supplementation but not by low (none) level or medium (200 μg/g) level zinc supplementation. Increased kidney copper concentrations have been reported associated with administration of cadmium in rats (Petering et al., 1979b; Stonard and Webb, 1976) and lambs (Doyle and Pfander, 1975). This effect was not found in calves in this study. On the contrary, the highest kidney copper concentration was observed in high level zinc calves which, according to analytical results for liver, kidney, muscle and blood, had the lowest cadmium body burden.

E. Muscle

Muscle cadmium concentrations (Table 12) of calves fed 50 μg/g cadmium for 60 days in this study were significantly (p < 0.05),
progressively decreased by 200 and by 600 μg/g of supplemental dietary zinc. Cadmium distribution to muscle in the bovine is reported to be only 0.7% and 1.4% that of the kidney and liver, respectively (Neathery et al., 1974). For the low level zinc calves in this study, the muscle concentration of cadmium was 0.04% and 0.18% that of kidney and liver, respectively. Cadmium fed low level zinc calves had a muscle cadmium concentration only 4 times greater than that of baseline calves. Muscle, with a ration cadmium concentration to muscle cadmium concentration ratio of 3,531, is a much less sensitive tissue than kidney or liver for estimating cadmium body burden in the bovine.

Muscle zinc concentrations (Table 12) of calves fed 50 μg/g cadmium for 60 days were not significantly (p < 0.05) affected by the level of supplemental dietary zinc. Unlike liver and kidney in this study which demonstrated apparent breakdown in zinc homeostasis with resultant elevation of tissue zinc concentrations at the high level of zinc supplementation, muscle zinc concentrations were unchanged in agreement with the observations of Miller et al. (1970). Muscle tissue would therefore be of no value as an indicator of elevated dietary zinc levels.

The muscle copper concentrations (Table 12) of calves in this study did not appear to respond to either cadmium or zinc feeding. Cadmium fed calves did not have muscle copper levels different from those of baseline calves and there was no significant (p < 0.05) difference in muscle copper concentrations among zinc supplementation levels.
F. Hair

Hair cadmium (Table 13), zinc (Table 14) and copper (Table 15) concentrations of calves fed 50 µg/g cadmium for 60 days showed no significant (p < 0.05) differences among zinc level supplementation groups. The observation that not previously clipped hair had even higher concentrations of cadmium and zinc than previously clipped 60 day post-cadmium feeding hair samples (Appendix C) strongly suggests an exogenous source and places doubt on the value of hair samples in predicting dietary or tissue levels of these 2 elements.

Dorn et al. (1974a) and Nishiyama and Nordberg (1972) reported that hair absorbs cadmium from environmental sources of contamination which is not readily removed by washing and which may not accurately represent body accumulation.

Julshamn et al. (1977) found no significant increase in rat hair cadmium concentration following 9 weeks of feeding 100 µg/g cadmium. Calves in this study occasionally became soiled with feces and were exposed to dust from dried feces and experimental ration, both of which were very high in cadmium. Apparently, cadmium was absorbed from exogenous sources by old hair and presumably by the regrowth hair which was not effectively removed by washing. Therefore, in this study, the 5 to 9 fold increase in hair cadmium concentrations observed over the 60 day feeding period in calves fed 50 µg/g cadmium cannot with certainty be ascribed to endogenous cadmium.
G. Blood

Calves fed 50 µg/g cadmium showed gradually increasing blood cadmium concentrations over 60 days reaching, for non-zinc supplemented calves, a level of 10.6 times the pre-cadmium feeding level. From 30 through 60 days, calves fed cadmium plus 200 and 600 µg/g supplemental zinc had significantly (p < 0.05) lower blood cadmium concentrations than non-zinc supplemented calves (Table 16). Blood cadmium concentration does reflect increased dietary cadmium but apparently only after a period of time sufficient for an increase in the body burden of cadmium to occur.

Within 3.5 days after starting to feed 50 µg/g cadmium and 3 levels of zinc, blood zinc concentrations (Table 17) were significantly (p < 0.05) increased by the feeding of 600 µg/g of supplemental zinc. Blood zinc concentrations were not affected by 200 µg/g supplemental zinc. Apparently, blood zinc homeostasis is overcome at dietary zinc levels between 200 and 600 µg/g where a blood zinc response to dietary zinc begins to be manifested.

The feeding of 200 µg/g and 600 µg/g of supplemental zinc had no significant (p < 0.01) effect on blood copper concentrations (Table 18) of calves fed 50 µg/g cadmium over 60 days.

H. Feces

Supplemental zinc feeding resulted in no change at most sampling times (significance, p < 0.05) in the fecal cadmium concentration among groups of calves fed 50 µg/g cadmium or cadmium plus zinc. These fecal cadmium data were not nearly sensitive enough, considering an expected
gastointestinal absorption of only approximately 5% (Doyle et al., 1974), to attempt to reveal differences in the rate of cadmium absorption among zinc level groups of calves.

If such differences in cadmium absorption could be determined, the question might be answered as to whether zinc-influenced cadmium absorption or zinc-influenced cadmium metabolism was responsible for the reduction in cadmium body burden by supplemental zinc. Total collection of feces and perhaps urine in metabolism study stalls would be required to collect highly accurate cadmium excretion data.

Fecal zinc concentrations reflected the relative levels of supplemental zinc fed and were significantly (p < 0.01) different for all treatment groups from 1.5 days through 60 days.

The decrease in fecal copper concentrations observed at 1.5 days appears to be associated with the start of cadmium feeding and further, it appears that the decrease was ameliorated by the high level of zinc supplementation. This would indicate an apparent increase in copper absorption and would differ from the finding by Davies and Campbell (1977) that cadmium decreased the intestinal absorption of copper in rats. Another possible explanation for the observed decrease in fecal copper concentrations is that it could reflect a change in dietary copper concentration which might have been lower in basal and experimental rations than in the hay fed during the adjustment period.

Fecal cadmium and zinc levels reflected closely the dietary levels of cadmium and zinc. Fecal cadmium and zinc concentrations reached
essentially maximum and plateau levels by 3.5 days after calves were
started on experimental rations (Figures 5 and 6). Therefore, fecal
cadmium and zinc concentrations appear to be excellent indicators of
dietary cadmium and zinc concentrations.

In addition, it would seem possible to actually estimate the
dietary concentrations by using the feces concentration to ration
concentration proportion for each element as calculated and shown in
Table 22. At a dietary level of 49 μg/g cadmium, calves' fecal cadmium
concentrations were 3.4 times the ration cadmium concentration. Calves
being fed low level zinc (56 μg/g), medium level zinc (260 μg/g) and
high level zinc (675 μg/g) had fecal concentrations 2.8, 2.4 and 2.1
times the ration zinc concentrations, respectively. Fecal copper
concentrations were 3.3 times higher than the 7.3 μg/g ration copper
concentration.

Feces contain higher concentrations of cadmium, zinc and copper
than rations. This occurs even though there are absorption
subtractions and is probably because these elements are also mainly
excreted in feces and because there is a net reduction in dry matter
quantity during digestion.
VI. SUMMARY

The increasing contamination of the environment with the toxic heavy metal cadmium presents the hazard of cadmium being translocated from crops and livestock into human foods. Cadmium causes decreased growth and productivity, renal damage, immunosuppression and teratologic effects in animals. In humans, cadmium has been associated with cardiovascular disease, renal and prostatic cancer, emphysema, renal dysfunction and osteomalacia.

Cadmium and zinc, an essential element for plants, animals and humans, occur together in nature and have metabolic interactions with each other and with copper in animals. The metabolism of cadmium, zinc and copper involves metallothioneins which are specialized low molecular weight proteins capable of binding these elements. Cadmium acts as a metabolic antagonist to zinc and causes increased liver and kidney zinc concentrations when fed to animals receiving normal dietary levels of zinc.

The effect of supplemental dietary zinc on the concentrations of cadmium, zinc and copper in the liver, kidney cortex, muscle, hair, blood and feces of calves fed 50 µg/g cadmium for 60 days was studied.

Four calves from each of 4 sewage sludge receiving farms and 4 calves from each of 4 farms not exposed to sewage sludge were randomly allocated into 4 different experimental groups. The groups, each
containing 1 calf from each farm, consisted of a baseline group, treatment group 1 which was fed 50 μg/g cadmium, treatment group 2 which was fed 50 μg/g cadmium plus 200 μg/g zinc, and treatment group 3 which was fed 50 μg/g cadmium plus 600 μg/g zinc.

At the time calves were received from the farms, hair samples were taken and the baseline group calves were slaughtered for liver, kidney and muscle samples. Blood and feces samples were collected from the calves of the other 3 groups and they were then started on the appropriate cadmium or cadmium plus zinc ration. Samples of blood and feces were collected at 0.5, 1.5, 3.5, 8, 15, 30, 45 and 60 days after starting to feed cadmium. At 60 days, the calves were slaughtered and samples of liver, kidney, muscle and hair were collected.

All samples were analyzed for cadmium, zinc and copper by atomic absorption spectroscopy after digestion with hot concentrated nitric acid. The method of standard additions was used for calibration.

The cadmium concentrations of all sample types collected were markedly increased by the feeding of cadmium. The cadmium concentrations of liver, kidney cortex, muscle and blood of calves supplemented with 600 μg/g zinc were significantly (p < 0.05) less than those of calves not supplemented with zinc. Feeding 600 μg/g supplemental zinc significantly (p < 0.05) increased zinc concentrations of liver, kidney cortex, blood and feces of calves fed 50 μg/g cadmium for 60 days. Supplemental zinc did not change the cadmium concentration in hair or feces or the zinc concentrations in muscle or hair of these calves.
The copper concentrations of liver and kidney cortex were significantly (p < 0.05) increased by 600 μg/g of supplemental zinc in calves fed 50 μg/g cadmium for 60 days. Feeding cadmium or cadmium plus zinc did not significantly (p < 0.05) change the copper concentrations of muscle, hair, blood or feces.

Supplemental zinc, either through a metabolic displacement of cadmium or by an interference with cadmium absorption, was effective in reducing by one half the accumulation of cadmium in the edible tissues, liver, kidney and muscle of calves fed a high level of cadmium. Therefore, levels of 200 (minimal effect) to 600 μg/g zinc in the ration of cadmium exposed cattle would reduce the deposition of cadmium in liver, kidney and muscle. Further work should examine the usefulness of using zinc as a therapeutic agent in cattle with high tissue cadmium levels.

Liver and kidney but not muscle or hair cadmium concentrations were sensitive indicators of dietary cadmium exposure. Blood cadmium concentrations reflected an increased cadmium body burden. Liver, kidney and blood but not muscle or hair zinc concentrations reflected only dietary zinc concentrations high enough (600 μg/g) to overcome zinc homeostasis. Feces cadmium, zinc and copper concentrations could be used to estimate dietary cadmium, zinc and copper concentrations, respectively.
APPENDIX A

Calves (Identified by Ear Tag Numbers) Randomly Allocated to Treatment Groups from Sludge Exposed and Non-Sludge Exposed Farms

<table>
<thead>
<tr>
<th>Farm No.</th>
<th>Sludge Exposed</th>
<th>Non-Sludge Exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1</td>
</tr>
<tr>
<td>3005</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>4018</td>
<td>38</td>
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<td>60</td>
</tr>
<tr>
<td>4017</td>
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<td>54</td>
</tr>
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<td>4509</td>
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</tr>
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<td>4519</td>
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<td>56</td>
<td>57</td>
</tr>
<tr>
<td>4516</td>
<td>65</td>
<td>66</td>
</tr>
</tbody>
</table>

*a Baseline calves were not fed experimental rations and were sampled when received from farms. Group 1, 50 µg/g cadmium; group 2, 50 µg/g cadmium plus 200 µg/g zinc; and group 3, 50 µg/g cadmium plus 600 µg/g zinc added to ration for 60 days.*
## APPENDIX B

Cadmium Concentrations in Liver and in Kidney Cortex of Male and Female Calves Fed 50 ug/g Cadmium and 3 levels of Zinc for 60 days

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Treatment Group</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>1</td>
<td>7.97 ± 0.53</td>
<td>9.56 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.79 ± 2.04</td>
<td>6.73 ± 0.81</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.66 ± 0.66</td>
<td>5.12 ± 1.30</td>
</tr>
<tr>
<td>Kidney</td>
<td>1</td>
<td>36.0 ± 6.8</td>
<td>36.8 ± 6.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>22.0 ± 2.5</td>
<td>30.6 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10.1 ± 1.4</td>
<td>18.8 ± 5.8</td>
</tr>
</tbody>
</table>

\* Means of male and female calves were not significantly (p < 0.05) different (Student's t test) for any treatment group for either tissue.

\* Group 1, 50 µg/g cadmium; group 2, 50 µg/g cadmium plus 200 µg/g zinc; and group 3, 50 µg/g cadmium plus 600 µg/g zinc added to ration for 60 days.
APPENDIX C

Cadmium (Cd), Zinc (Zn) and Copper (Cu) Concentrations (µg/g) in Previously Clipped and Not Previously Clipped Hair of Calves Fed 50 µg/g Cadmium and 3 Levels of Zinc for 60 Days

<table>
<thead>
<tr>
<th>Element</th>
<th>Treatment Groupa</th>
<th>Previously Clippedb</th>
<th>Not Previously Clipped</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>1</td>
<td>0.94</td>
<td>2.26</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.97</td>
<td>1.78</td>
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<td></td>
<td>3</td>
<td>0.76</td>
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<tr>
<td>Zn</td>
<td>1</td>
<td>135</td>
<td>172</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>145</td>
<td>185</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>151</td>
<td>192</td>
</tr>
<tr>
<td>Cu</td>
<td>1</td>
<td>7.72</td>
<td>10.75</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.19</td>
<td>10.94</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>9.18</td>
<td>14.44</td>
</tr>
</tbody>
</table>

a Each group contained 8 calves. Group 1, 50 µg/g cadmium; group 2, 50 µg/g cadmium plus 200 µg/g zinc; and group 3, 50 µg/g cadmium plus 600 µg/g zinc added to ration for 60 days.

b Means of treatment groups were not significantly (p < 0.05) different.
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