# Effect of Chemical Form of Orally Administered $^{65}Zn$ on Absorption and Metabolism in Cattle[1] (36274)

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Zinc absorption and metabolism have been reviewed recently (1, 2). Metabolism of $^{65}Zn$ in ruminants has been studied with tracer doses of $^{65}ZnCl_2$ (3–5). Whether zinc added to the diet as the chloride is absorbed and metabolized to the same degree and in the same way as that existing in natural feedstuffs has not been investigated in the ruminant. The objective of this work was to study the absorption and tissue distribution in the calf of $^{65}Zn$ as it exists in natural plant materials (forage) in comparison with $^{65}Zn$ as $^{65}ZnCl_2$. It was considered important to have identical diets so that the forage could not have an effect separate and apart from the chemical form and complexes associated with zinc. To accomplish this it was deemed essential to use only a tracer amount of forage. This required development of a procedure for incorporating a sufficiently high concentration of $^{65}Zn$ into forage.

*Materials and Methods.* A composite of forage material containing $^{65}Zn$ was used for the animal metabolism studies. It consisted of leaves and stems from corn plants grown for 19 or 33 days in 1.82 kg of sand media/pot. Each pot was dosed with 265 or 530 $\mu$Ci of $^{65}Zn$ at planting. The $^{65}Zn$ forage was ground and thoroughly mixed before it was administered orally to calves.

Six uncastrated male Holstein calves (av 67 days of age and 65.5 kg) were given either $^{65}Zn$ in forage or $^{65}Zn$ as $^{65}ZnCl_2$ orally via gelatin capsules in a single dose. The three calves given $^{65}Zn$-forage received 10.2 g of forage dry matter containing 35 $\mu$Ci of $^{65}Zn$. Likewise, a tracer dose of $^{65}ZnCl_2$ (sp act 4.5 Ci of $^{65}Zn$/g of Zn) given to three calves was in HCl solution buffered to a pH of 4.73 with sodium acetate buffer and adsorbed on facial tissue in a gelatin capsule. These calves were given 10.2 g of comparable but nonradioactive forage via capsule; and those given radioactive forage received a capsule of facial tissue.

The calves were fed a zinc-deficient purified diet (2.7 ppm zinc) *ad libitum* for 7 days before dosing and at 2.5% of body weight daily during a 7-day total fecal collection period after dosing. Average daily feed and zinc intakes were 1511 g and 4.2 mg.

The diet had the following composition (kg/100 kg): glucose monohydrate, 20.5; cornstarch, 25.0; dried whole whey (spray process), 20.0; cellulose, 10.0; gelatin (flake, 50-bloom), 13.0; urea (feed grade, 42% N), 0.5; $KHCO_3$, 1.0; $NaHCO_3$, 2.0; dicalcium phosphate (anhydrous, food grade), 2.0; $CaCO_3$ (marble dust), 1.0; lard (stabilized) 3.0; and (g/100 kg): $Na_2SO_4$ (anhydrous), 350; KCl, 560; NaCl, 484; MgO (56% Mg), 165; $Fe_2(SO_4)3{\cdot}\eta H_2O$ (20% Fe by assay), 22; $MnSO_4{\cdot}H_2O$, 11; $CuSO_4$, 3.1; $CoCO_3$ (45–50% Co assay), 0.066; KI, 0.036; menadione sodium bisulfite (63%), 0.33; *d*-alpha tocopheryl acetate (333 IU of vitamin E activity/g), 2.2; vitamin $D_3$ (200,000 IU/g), 2.2; vitamin A palmitate (325,000 IU/g), 17.6; choline chloride (70%), 264; and oxytetracycline·HCl (5.5%), 88.

Total fecal excretion was collected daily, weighed, thoroughly mixed; and a sample

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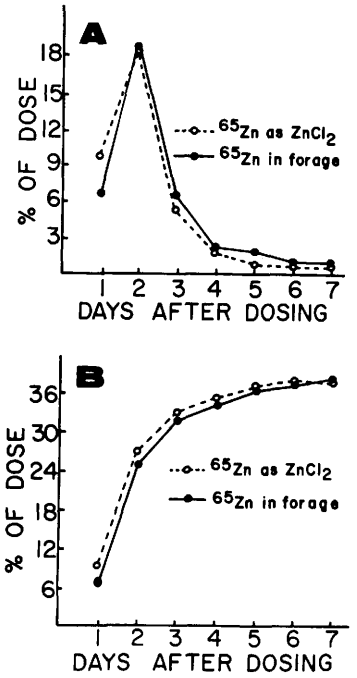


FIG. 1. Effect of chemical form of zinc (as $^{65}ZnCl_2$ vs $^{65}Zn$ in forage) on fecal $^{65}Zn$ excretion following a single oral dose: (A) daily rate; and (B) accumulated total (3 calves/treatment).

was dried for $^{65}Zn$ determination with an automatic gamma ray test tube changer system with a NaI(TI) well-type crystal.

Seven days after dosing, the calves were anesthetized by an intravenous injection of sodium pentobarbital and killed immediately by cannulation of the carotid artery to remove blood from organs and tissues. Organs and tissues were removed and sampled as follows: (a) liver, from lower center of reticular impression area; (b) kidney, whole organ after removal of visceral layer; (c) spleen, whole organ; (d) lung, center cross section area; (e) heart, apex; (f) duodenum, first 1.8 m of small intestine; and (g) small intestine, second 1.8 m of small intestine. Each small intestine section was washed twice with 0.9% saline solution. Samples were frozen until analyzed for $^{65}Zn$ content.

*Results.* Both daily rate and accumulated total fecal $^{65}Zn$ excretion for calves given $^{65}Zn$ in forage and as the chloride were very similar (Fig. 1a and b). Excretion rate peaked on the second day after dosing, followed by a sharp decrease through day 4 and a slower decline thereafter (Fig. 1a). Total fecal $^{65}Zn$ excretion for the 7-day collection period averaged 38% for each of the chemical forms of $^{65}Zn$ (Fig. 1b). Net $^{65}Zn$ absorption (calculated by difference between fecal excretion and 100%) was 62% of the dose for each treatment.

Even though net absorption was the same for each treatment, $^{65}Zn$ retention in all the soft tissues sampled was considerably higher in those given $^{65}Zn$ as forage (Table I). This effect (percentage of $^{65}Zn$ dose per kilogram of fresh tissue) was significant ($p < .05$) in liver, spleen, heart, duodenum, lung, and small intestine but not in the kidney. However, when $^{65}Zn$ concentration was expressed as percentage of absorbed dose, kidneys from calves dosed with $^{65}Zn$ in the forage contained more $^{65}Zn$ ($p < .10$). For both groups, the liver contained the highest level of $^{65}Zn$ followed by spleen, kidney, heart, small intestine, duodenum, and lung (Table I).

*Discussion.* Previous studies suggested but did not prove that wide differences in zinc availability for ruminants may exist with zinc in different chemical forms (1, 2). For ex-

TABLE I. Effect of Chemical Form of $^{65}Zn$ Distribution in Tissues of Normal Calves Fed a Zinc-Deficient Diet.

| Tissue | $^{65}Zn$ (% of $^{65}Zn$ dose/kg of fresh tissue) | |
|---|---|---|
| | As $^{65}ZnCl_2$ | In forage |
| Liver | 3.20 ± 0.35[c] | 4.64[d] ± 0.38[c] |
| Spleen | 2.51 ± 0.33 | 3.47[d] ± 0.07 |
| Kidney | 2.24 ± 0.29 | 2.91[e] ± 0.07 |
| Heart | 2.00 ± 0.21 | 2.79[d] ± 0.09 |
| Lung | 1.49 ± 0.14 | 2.16[d] ± 0.12 |
| Duodenum[a] | 1.89 ± 0.24 | 2.60[d] ± 0.05 |
| Small intestine[b] | 1.95 ± 0.18 | 2.75[d] ± 0.08 |

[a] First 1.8 m of small intestine.
[b] Second 1.8 m of small intestine.
[c] Standard error of mean (3 calves/treatment).
[d] Significant at the 5% probability level.
[e] When data were calculated as percentage of absorbed dose/kilogram of fresh tissue, this difference was significant at the 10% probability level.

ample, this could explain the very wide differences observed in zinc requirements in different studies (1, 2). Also, such effects are well known in monogastric species (1, 2). The lack of differences in fecal $^{65}Zn$ excretion indicates that availability and absorption of $^{65}Zn$ as corn forage and as $^{65}ZnCl_2$ were comparable. However, these data do not preclude the possibility of wide differences in other natural feeds. The zinc-deficient diet would result in much higher zinc absorption than would be observed with practical diets, thus providing a critical test of the availability of $^{65}Zn$ for absorption (3, 6–8).

With fecal $^{65}Zn$ excretion very similar in calves dosed with $^{65}Zn$ forage and $^{65}ZnCl_2$, total absorption and total retention had to be comparable. The higher $^{65}Zn$ concentrations in metabolically more active tissues of calves given $^{65}Zn$ as forage must have been offset by lower concentrations in tissues not analyzed. These probably included bone and possibly muscle. Earlier, it was shown that, in zinc-deficient animals, $^{65}Zn$ concentrations are increased in most soft tissues but that an opposite effect occurs in bone (2, 3, 6, 9, 10). The tissues taken for analysis were those which are metabolically more active for $^{65}Zn$ (1, 2, 4, 9). $^{65}Zn$ accumulation following an oral dose is much slower in tissues such as red blood cells, muscle, and bone, so that much lower concentrations would have been expected in these 7 days after dosing (9). However, since the total amount of such tissues is far greater than that of those studied, the reduction would not have had to be large to offset the increase observed in the tissues studied.

Since the animals receiving the two forms of $^{65}Zn$ were comparable in all other respects, it is apparent that $^{65}Zn$ metabolism must have been different after absorption. Zinc occurs in the animal and is transported almost entirely combined with organic compounds (2, 11–13). In absorption and transport, apparently zinc is passed from one binding protein to another (2, 14). It may pass from one protein to another as a metallo-ligand complex with compounds such as ethylenediamine tetraacetate (EDTA) or amino acids serving as the nonprotein ligands (14–16). The differences observed in this study may have been due to $^{65}Zn$ in the two forms being combined and transported in combination with different ligands, causing a substantial difference in the way the zinc was metabolized. The metabolic difference could have been mediated through differences in the relative affinity of the various tissues for this zinc compared to the other dietary zinc. Such a concept provides a credible explanation for the influence of other compounds, such as histidine (14, 17), on the alleviation of some zinc-deficiency symptoms but not others (14, 17). Also, this evidence and such a concept suggest that mineral metabolism is much more complex than is generally believed.

*Summary.* Absorption and metabolism of $^{65}Zn$ in the natural chemical form of forage and as $^{65}ZnCl_2$ were studied in Holstein calves following single tracer oral doses. Chemical form did not affect $^{65}Zn$ excretion or total retention but $^{65}Zn$ was higher in calves given $^{65}Zn$ as forage in metabolically more active tissues (liver, spleen, heart, lungs, duodenum, and small intestines, $p < .05$). Thus, chemical form of $^{65}Zn$ dose affected tissue distribution but not absorption. It is theorized that $^{65}Zn$ in the two chemical forms was metabolized differently after absorption due to different $^{65}Zn$–ligand combinations being transported from the intestine to the tissues.

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