Effect of Zinc Source and Dietary Level on Zinc Metabolism in Holstein Calves*

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ABSTRACT

Forty-eight Holstein male calves were stratified by origin and body weight and randomly assigned to one of 4 treatment groups. Dietary treatments were administered in 2 phases. In phase 1, treatment groups received the basal diet with no supplemental Zn (control), basal diet plus 20 mg of Zn/kg of DM as ZnSO₄ or Zn proteinate (ZnProt), or basal diet plus 20 mg of Zn/kg of DM with 50% of the Zn supplied from each source (ZnM) for 98 d. In phase 2, calves continued to receive the same Zn source fed in phase 1; however, half of the calves in each treatment group were randomly selected to receive 500 mg of Zn/kg of DM (HiZnSO₄, HiZnProt, HiZnM) for 14 d. Gain, feed intake, and feed efficiency of calves were not affected by treatment in either phase of the experiment. Treatment had no effect on plasma Zn concentration or alkaline phosphatase activity in phase 1, but liver Zn concentration was greater in calves fed ZnSO₄ than those fed ZnProt. In phase 2, plasma Zn was greater in calves fed HiZnProt and HiZnM than in those fed HiZnSO₄. Liver Zn was greater in calves fed HiZnProt than in those fed HiZnSO₄. Duodenal Zn concentrations were greater in calves supplemented with HiZnProt and HiZnM than those supplemented with HiZnSO₄. Liver metallothionein was greater in calves that received 500 mg of Zn/kg than in calves that received 20 mg of Zn/kg, but was not affected by Zn source. Calves fed HiZnProt and HiZnM had greater kidney Zn concentrations than those fed HiZnSO₄. Heart, spleen, testicular, and bone Zn concentrations were not affected by Zn source. Hoof wall samples contained nearly 3-fold greater Zn concentrations than hoof sole. Calves fed ZnSO₄ had greater Zn concentration in hoof wall samples than those fed ZnM. Hoof sole Zn concentration was not affected

by Zn source or concentration. Plasma and tissue Zn concentrations at harvest were generally similar in calves supplemented with 20 mg of Zn/kg from ZnSO₄ or ZnProt. However, when supplemented at 500 mg of Zn/kg, ZnProt was absorbed to a greater extent than ZnSO₄, based on higher plasma, liver, duodenal, and kidney Zn concentrations.

(Key words: zinc, proteinate, bioavailability, cattle)

Abbreviation key: Alp = alkaline phosphatase, HiZnM = high zinc mix, HiZnProt = high zinc proteinate, HiZnSO₄ = high zinc sulfate, MT = metallothionein, ZnM = zinc mix (50% ZnSO₄, 50% ZnProt), ZnProt = zinc proteinate.

INTRODUCTION

Metal proteinates are produced by chelation of a soluble metal salt with amino acids and/or partially hydrolyzed protein (AAFCO, 2000). Previously, Zn proteinate (ZnProt) improved performance and certain carcass characteristics in finishing steers (Spears and Kegley, 2002), increased force required for shearing of hooves in heifers (Reiling et al., 1992), improved hoof quality scores (Kessler et al., 2003), and increased Zn retention in lambs (Lardy et al., 1992) relative to inorganic Zn sources (ZnSO₄ or ZnO). Lambs supplied with high dietary Zn concentrations (1400 mg of Zn/kg of DM) from ZnProt had greater tissue concentrations of Zn than those supplemented with ZnSO₄ (Cao et al., 2000).

While evidence suggests that organic Zn sources can, under certain conditions, enhance performance, and improve health and reproduction, specific mechanisms underlying observed responses are unclear (Spears, 1996). Apparent absorption of Zn has been similar in ruminants supplemented with organic Zn or inorganic Zn (Spears, 1989; Lardy et al., 1992; Nockels et al., 1993). However, limited research suggests that postabsorptive metabolism of organic trace minerals may differ from inorganic forms (Spears, 1989; Eckert et al., 1999). The present study was conducted to determine the effects of Zn source (ZnSO₄ vs. ZnProt) on metabolism and tissue concentrations of Zn in calves fed normal or high concentrations of Zn.

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Table 1. Ingredient composition of basal diet.¹

Ingredient	% of DM
Cottonseed hulls	40.00
Cracked corn	45.08
Soybean meal	11.18
Calcium carbonate	1.40
Urea	1.00
Calcium sulfate	0.60
Dicalcium phosphate	0.50
Sodium chloride	0.20
Vitamin premix ²	0.02
Mineral premix ³	0.02

¹Basal diet analyzed 28 mg of Zn/kg of DM.

²Vitamin premix contained per kg: 6,600,000 IU of vitamin A, 4,400,000 IU of vitamin D, and 2,200 IU of vitamin E.

³Trace minerals provided in mg/kg of diet: 25 mg of Fe as FeSO₄, 20 mg of Mn as MnSO₄, 10 mg of Cu as CuSO₄, 0.5 mg of I as Ca(IO₃)·H₂O, 0.1 mg of Co as CoCO₃, and 0.1 mg of Se as NaSeO₃.

MATERIALS AND METHODS

Care and handling of the animals and sampling procedures described herein were approved by the North Carolina State University Animal Care and Use Committee.

Forty-eight male Holstein calves were obtained from research dairies in the North Carolina Department of Agriculture and North Carolina State University systems. Due to age differences, one group of calves ($n = 16$; average BW = 183.5 kg) started on the study 28 d before the remaining calves ($n = 32$; average BW = 148.1 kg). Start date did not affect ($P > 0.10$) any measured variables; thus, data were pooled for statistical analysis and will be referred to as one experiment.

Calves were stratified by origin and BW and randomly assigned to one of 4 treatment groups. Dietary treatments were administered in 2 phases. In phase 1 (d 0 to 98), treatment groups received the basal diet with no supplemental Zn (control), basal diet plus 20 mg of Zn/kg of DM as ZnSO₄ or ZnProt (Chelated Minerals Corporation, Salt Lake City, UT) or basal diet plus 20 mg of Zn/kg of DM with 50% of the Zn supplied from each source (**ZnM**). In phase 2 (d 99 to 112) cattle continued to receive the same Zn source fed in phase 1; however, half of the calves in each treatment group were randomly selected to receive 500 mg of Zn/kg of DM (**HiZnSO₄**, **HiZnProt**, **HiZnM**). Calves were fed a corn-soybean meal-cottonseed hull basal diet (28.0 mg of Zn/kg of DM; Table 1) and were housed 2 per pen in covered, slotted-floor pens (3 × 4 m). The basal diet was formulated to meet or exceed nutrient requirements for growing calves (NRC, 1996), with the exception of Zn. Weights were taken prior to feeding on d -1, 0, 28, 56, 84, 98, 111, and 112. On d 112, all calves were transported to a commercial abattoir for slaughter.

Blood samples were collected on d 0, 28, 56, 84, 98, and 112 via jugular venipuncture in heparinized-trace

mineral free tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Whole blood was centrifuged at $1760 \times g$ for 15 min at 4°C, then plasma was aspirated into 5-mL polyethylene tubes (Elkay Products Incorporated, Shrewsbury, MA) and frozen at -20°C until analysis. Plasma alkaline phosphatase (**Alp**) activity was determined by a kinetic assay (ALP 20, Sigma Diagnostics Incorporated, St. Louis, MO).

Liver biopsies were collected on d 0, 56, and 98. Biopsy sites were clipped of hair, scrubbed 3 times with betadine (Purdue Frederick, Norwalk, CT) and 70% ethyl alcohol. A small incision was made between the 11th and 12th ribs, on a line from the tubercosae to the point of the shoulder. Liver tissue was removed using a Jamshide bone marrow punch (0.4 cm in diameter × 10 cm in length; Allegiance Healthcare Corp., McGaw Park, IL) while applying suction with a 10-mL syringe. Liver samples were immediately rinsed with 0.01 mol/L PBS (pH 7.4) and drained to remove contaminating blood. Samples were then transferred to acid-washed 5-mL polyethylene tubes, capped, and placed on ice for transport to the laboratory, where they were stored at -20°C until analysis.

Hair samples were collected by clipping a 4 × 4 cm area 56 d prior to, and the day before, slaughter. Samples were stored in plastic bags (Whirl-Pak, Nasco, Fort Atkinson, WI) at room temperature until analysis. Hair samples were washed with 0.1 M SDS solution and rinsed repeatedly with deionized water prior to analysis. Heart, kidney, liver, omasum, rumen, spleen, duodenum samples, and the right front leg (below the knee) of each calf were collected at the abattoir and transported on ice to the laboratory, where they were stored at -20°C. Duodenal segments (7 to 10 cm in length) were excised from an area approximately 30 to 60 cm from the pylorus, immediately rinsed and flushed with ice-cold 0.85% saline, and placed into ice-cold saline for transport to the laboratory. Upon arrival, segments were cut longitudinally to expose the mucosa and rinsed again with ice-cold saline to remove remaining digesta. Mucosal cells were then removed by scraping the tissue with a glass microscope slide. Cell scrapings were transferred into preweighed 50-mL centrifuge tubes (Sorvall, Kendro Laboratory Products, Newtown, CT), weighed, and diluted 1:4 (wt/vol) with glycine buffer containing 0.2 mmol phenylmethylsulfonyl fluoride, 0.6 mg of leupeptin, 0.9 mg of pepstatin A, and 0.2 mg of sodium azide/L (pH 8.6). Samples were then homogenized (Polytron, Brinkmann Instruments, Westbury, NY), and centrifuged at $20,000 \times g$ for 30 min (Sorvall RC5C, Kendro Laboratory Products, Newtown, CT). Supernatant fractions were collected, heated for 2 min at 100°C, and centrifuged again at $20,000 \times g$ for 30 min. Final supernatant fractions were then transferred to 15-mL screw-top tubes (Falcon,

Becton Dickinson, Franklin Lakes, NJ) and stored at -20°C until analyzed for Zn. Samples (approximately 2 g) of ruminal and omasal epithelial tissue were diluted 1:4 (wt/vol) with 0.5 mol/L glycine buffer (pH 8.3), homogenized, and heated for 2 min at 100°C . Samples were then centrifuged at $25,000 \times g$ for 2 min and supernatant fractions stored at -20°C for Zn analysis. The procedure used to prepare duodenal, ruminal, and omasal tissues should have removed loosely bound Zn and Zn bound to metallothionein (MT). Zinc tightly bound to proteins and metalloenzymes would not be in the supernatant fraction.

Bone, hoof, and skin samples were harvested from the right front leg of each calf. Hoof tissue was collected by first removing a thin layer of tissue to remove contamination. Then a 0.5-cm slice was cut from each digit parallel to the sole of the hoof. Each hoof sample was washed with 1.0 M SDS, rinsed repeatedly with deionized water, and separated by location on the hoof (wall or sole). Bone (metacarpal), and skin samples were removed from 1.0-cm slices cut perpendicular to the longitudinal axis of the leg, both in the center of the shaft and approximately 5 cm from the distal end of the metacarpal bone. Hair was shaved from each skin sample, and visible connective tissue was removed from the skin.

Bone samples were processed as described by Armstrong et al. (2000). Briefly, bone cross sections were weighed and dried for 48 h at 100°C , then weighed again to determine DM. Samples were then wrapped in filter paper, placed in a side-arm Soxhlet extraction apparatus, extracted with petroleum ether for 48 h and allowed to air dry under a hood for 48 h. After lipid extraction, bone sections were dried at 100°C for 18 h and weighed for DM determination. Bone samples were then ashed in a muffle furnace at 600°C for 48 h. Bone ash was dissolved in 10 mL of 6 N HCl and brought to 25 mL with deionized water for Zn analysis.

Feed and tissue samples (except duodenal, ruminal, omasal, and bone) were dried at 100°C for 48 h, weighed, and wet ashed using a microwave digestion (model MDS-81D, CEM, Matthews, NC) procedure described by Gengelbach et al. (1994). Plasma and tissue homogenates (rumen, omasum, and duodenum) were diluted 1:4 with 5% HNO_3 and centrifuged at $1760 \times g$ for 15 min immediately prior to Zn determination. Ashed tissue and feed samples and plasma and tissue homogenate supernatant fractions were analyzed for Zn content by flame atomic absorption spectroscopy (model AA-6701F, Shimadzu, Kyoto, Japan).

Liver MT concentration was determined on liver samples using a nonradioactive Ag binding assay procedure (Lee et al., 1989) as modified by Carlson et al. (1999). Silver in the supernatant fraction was determined by flame atomic absorption spectroscopy.

Statistical Analysis

Statistical analysis of liver and plasma Zn concentrations and plasma Alp activity in phase 1 were analyzed as repeated measures using the Mixed procedure of SAS as described by Littell et al. (1998). Animal within treatment was used as a random error term. The model included treatment, time, and treatment $\times$ time interaction. Initial values for plasma and liver Zn and plasma Alp were used as a covariant in the repeated measures analysis. Single degree of freedom contrasts were used to compare means between control vs. Zn-supplemented treatments, ZnSO_4 vs. ZnProt, and ZnSO_4 vs. ZnM.

Statistical analysis of performance data from phase 1 and all phase 2 data were performed by ANOVA using the general linear model procedure of SAS. The model contained treatment and error. Pen was used as the experimental unit for all performance data, while animal was used as the experimental unit for phase 2 plasma and tissue data. Single degree of freedom contrasts were used to compare means. Comparisons made were: control vs. 20 mg of Zn/kg treatments, high vs. low supplemental Zn, ZnSO_4 vs. ZnProt, HiZn SO_4 vs. HiZnProt, ZnSO_4 vs. ZnM, and HiZn SO_4 vs. HiZnM. Significance was declared at $P \leq 0.05$, and a trend was declared at $P \leq 0.10$.

RESULTS

Phase 1

Average daily feed intake, daily gain, and gain:feed were not affected by dietary treatment (Table 2). No treatment $\times$ time interactions were observed for plasma and liver Zn concentration or plasma Alp activity. Values shown in Table 3 are means across all sampling dates, adjusted using initial values as a covariant. Plasma Zn and Alp were not affected by Zn source or concentration. Liver Zn concentrations tended ($P < 0.10$) to be lower in control calves than in Zn-supplemented calves (Table 3). Calves supplemented with ZnSO_4 had greater ($P < 0.05$) liver Zn concentrations than those receiving ZnProt.

Phase 2

Average daily feed intake, daily gain, and gain:feed were not affected by dietary treatment (Table 2). However, calves supplemented with high dietary Zn levels tended ($P < 0.10$) to gain faster than those supplemented with the lower Zn levels during the 14-d period.

Zinc concentrations in duodenal mucosal scrapings from calves supplemented with HiZnProt and HiZnM were greater ($P < 0.01$) than those supplemented with HiZn SO_4 (Table 4). Zinc concentrations were greater ($P < 0.01$) in duodenal homogenates from calves supple-

Table 2. Effect of Zn source and level on performance.

	Treatment ¹							
Item	Control	ZnSO ₄	ZnProt	ZnM	HiZnSO ₄	HiZnProt	HiZnM	SE
Phase 1								
DMI, kg/d	7.4	7.0	7.1	7.0	...	...	...	0.4
Gain, kg/d	1.4	1.3	1.3	1.3	...	...	...	0.1
Gain:feed	0.19	0.19	0.18	0.18	...	...	...	0.01
Phase 2								
DMI, kg/d	9.2	7.0	8.7	7.8	8.8	8.1	8.9	0.7
Gain, kg/d	1.5	0.9	1.0	1.3	1.5	1.2	1.5	0.3
Gain:feed	0.16	0.13	0.15	0.12	0.18	0.19	0.13	0.02

¹Treatments consisted of 0 (control), 20 or 500 mg of supplemental Zn/kg of DM as ZnSO₄ (ZnSO₄ and HiZnSO₄), Zn proteinate (ZnProt and HiZnProt), or a mixture supplying 50% of supplemental Zn from each source (ZnM and HiZnM).

mented with high relative to low dietary Zn levels. Homogenates of ruminal and omasal epithelium were not affected by Zn source or concentration (Table 4).

Plasma Zn concentrations were greater ($P < 0.01$) in calves that received HiZnProt or HiZnM than in those fed HiZnSO₄ (Table 5). Calves supplemented with high dietary Zn had higher ($P < 0.01$) plasma Zn concentrations than those supplemented with 20 mg of Zn/kg.

Liver Zn was greater ($P < 0.01$) in calves fed high compared with low dietary Zn (Table 5). Calves fed HiZnProt tended ($P < 0.10$) to have greater liver Zn concentration than those receiving HiZnSO₄. Liver MT concentrations were nearly 50% greater ($P < 0.01$) in calves fed high dietary Zn levels relative to those fed low levels (Table 5). Zinc source did not affect liver MT concentrations.

Calves supplemented with HiZnProt and HiZnM had greater ($P < 0.01$ and $P < 0.05$, respectively) kidney Zn concentrations than those fed HiZnSO₄ (Table 6). Calves fed high dietary Zn levels had greater ($P < 0.01$) kidney concentrations than those supplemented with low Zn. Heart, spleen, testicle, and hair Zn concentrations were not affected by Zn source or concentration. Bone Zn concentration varied by location on the bone shaft. Zinc concentrations in bone slices harvested from the distal endplate region of the bone were 84.3% greater ($P < 0.01$) than slices harvested from the center of the shaft. Bone

shaft slices from calves supplemented with high dietary Zn had greater ($P < 0.05$) Zn concentrations than bone from calves supplemented at lower levels; however, bone Zn was not affected by Zn source.

At the low Zn concentration, calves fed ZnM had greater ($P < 0.05$) skin Zn concentrations than calves fed ZnSO₄ (Table 6). When fed high dietary Zn, calves fed HiZnSO₄ had greater ($P < 0.05$) skin Zn concentration than calves fed HiZnProt. As with bone Zn, hoof Zn concentration varied by location. Samples collected from the hoof wall contained nearly 3-fold greater ($P < 0.01$) Zn concentrations than samples taken from sole of the hoof (Table 6). Calves supplemented with ZnSO₄ had greater ($P < 0.05$) Zn concentration in hoof wall samples than calves supplemented with ZnM. Zinc concentration of hoof sole tissue was not affected by Zn source or concentration.

DISCUSSION

The addition of 20 mg of Zn/kg of DM to the control diet did not improve calf performance or increase plasma Zn concentration or plasma Alp activity. This suggests that the control diet was adequate in Zn to meet requirements of growing calves. The control diet analyzed 28 mg of Zn/kg, which approximates the NRC (1996) recommendation of 30 mg of Zn/kg. In previous studies (Spears,

Table 3. Effect of zinc source and level on plasma and liver zinc concentrations and plasma alkaline phosphatase activity (phase 1).

Item	Treatment				SE	Contrasts ¹
	Control	ZnSO ₄	ZnProt	ZnM		
Plasma Zn, mg/L	1.07	1.03	1.11	1.11	0.04	NS
Liver Zn, mg/kg DM	128.7	156.8	133.3	139.7	7.5	A [†] B*
Plasma Alp, U/L	99.2	106.9	103.3	100.5	4.7	NS

¹A = Control vs. Zn supplementation, B = ZnSO₄ vs. ZnProt.

²† $P < 0.10$.

³* $P < 0.05$.

Table 4. Effect of zinc source and level on tissue homogenate Zn concentration.

Item	Treatment ¹							SE	Contrast ²
	Control	ZnSO ₄	ZnProt	ZnM	HiZnSO ₄	HiZnProt	HiZnM		
	mg/L								
Duodenum	0.8	1.0	0.7	0.9	0.7	2.6	2.0	0.3	A**, B**, C**
Rumen	8.8	8.2	10.3	9.1	10.3	9.1	9.0	0.9	NS
Omasum	10.7	10.5	10.5	10.3	10.5	11.8	10.5	0.9	NS

¹Treatments consisted of 0 (control), 20 or 500 mg of supplemental Zn/kg of DM as ZnSO₄ (ZnSO₄ and HiZnSO₄), Zn proteinate (ZnProt and HiZnProt), or a mixture supplying 50% of supplemental Zn from each source (ZnM and HiZnM).

²A = Low Zn vs. high Zn, B = HiZnSO₄ vs. HiZnProt, C = HiZnSO₄ vs. HiZnM.

*P < 0.05.

**P < 0.01.

Table 5. Effect of zinc source and level on plasma and liver zinc concentrations and liver metallothionein (phase 2).

Item	Treatment							SE	Contrast ²
	Control	ZnSO ₄	ZnProt	ZnM	HiZnSO ₄	HiZnProt	HiZnM		
Plasma Zn (mg/L)	1.10	1.20	1.19	1.14	1.79	2.33	2.30	0.09	A**, B**, C**
Liver Zn (mg/kg DM)	133.2	127.9	146.1	175.9	231.0	331.0	286.8	32.9	A**, B†
Liver MT ((g/g tissue)	334.8	425.4	418.8	377.1	616.1	683.6	668.8	56.3	A**

¹Treatments consisted of 0 (control), 20 or 500 mg of supplemental Zn/kg of DM as ZnSO₄ (ZnSO₄ and HiZnSO₄), Zn proteinate (ZnP and HiZnProt), or a mixture supplying 50% of supplemental Zn from each source (ZnM and HiZnM).

²A = Low Zn vs. high Zn, B = HiZnSO₄ vs. HiZnProt, C = HiZnSO₄ vs. HiZnM.

†*P* < 0.10.

*P < 0.05.

**P < 0.01.

Table 6. Effect of zinc source and level on tissue zinc concentrations (phase 2).

Item	Treatment							SE	Contrast ²
	Control	ZnSO ₄	ZnProt	ZnM	HiZnSO ₄	HiZnProt	HiZnM		
	mg/kg DM								
Heart	102.8	80.1	85.3	89.6	90.2	90.7	102.1	12.1	NS
Kidney	95.8	97.2	89.7	114.3	183.1	356.7	299.5	33.5	A**, B**, C*
Spleen	112.5	97.6	97.6	92.8	105.2	99.3	99.3	9.0	NS
Testicle	98.3	83.7	77.5	69.4	84.1	83.2	104.9	11.4	NS
Skin	18.5	14.9	14.2	21.1	23.8	15.3	20.6	2.2	B*, D*
Hair	155.9	156.6	174.4	157.1	172.0	172.7	160.9	7.3	NS
Bone									
Shaft	49.7	45.6	44.6	49.5	55.2	53.8	59.5	4.7	A*
Endpoint	84.1	86.6	87.1	109.7	85.9	89.2	116.89	21.0	NS
Hoof									
Sole	61.6	58.7	66.7	54.3	51.5	54.9	51.3	5.5	NS
Wall	159.7	175.6	185.0	149.5	157.5	174.4	171.7	9.0	D*

¹Treatments consisted of 0 (Con), 20 or 500 mg of supplemental Zn/kg of DM as ZnSO₄ (ZnS and HiZnSO₄), Zn proteinate (ZnProt and HiZnProt), or a mixture supplying 50% of supplemental Zn from each source (ZnM and HiZnM).

²A = Low Zn vs. high Zn, B = HiZnSO₄ vs. HiZnProt, C = HiZnSO₄ vs. HiZnM, D = ZnSO₄ vs. ZnM.

*P < 0.05.

**P < 0.01.

1989; Spears and Kegley, 2002; Kessler et al., 2003), supplementing relatively low concentrations (10 to 25 mg/kg) of Zn to diets containing 24 to 35 mg of Zn/kg has not increased plasma Zn concentrations in cattle. Zinc concentrations in liver biopsy samples obtained on d 56 and 98 tended to be slightly lower in controls compared with calves supplemented with 20 mg of Zn/kg. However, Zn concentrations in tissues evaluated at the end of the 112-d study did not differ between controls and calves supplemented with 20 mg of Zn/kg. Holstein $\times$ Simmental calves fed a control diet (35 mg of Zn/kg) or the control supplemented with 10 mg of Zn/kg had similar Zn concentrations in muscle, liver, bone, and hair at the end of a 284-d study (Kessler et al., 2003). With the exception of rib cartilage and rumen, tissue Zn concentrations also were similar in lactating dairy cows fed diets containing 16.6 or 39.5 mg of Zn/kg for 42 d (Neathery et al., 1973).

Differences between Zn sources were generally not observed when Zn was supplemented at the low level. Liver Zn was lower in calves supplemented with ZnProt than in those receiving ZnSO₄ on d 56 and 98; however, Zn concentrations in liver samples obtained at harvest did not differ among Zn sources. Zinc concentrations in other tissues and plasma were similar in calves receiving ZnProt or ZnSO₄ at 20 mg of Zn/kg. Other studies (Rojas et al., 1996; Spears and Kegley, 2002; Kessler et al., 2003) have indicated no differences between organic and inorganic Zn sources in plasma or tissue concentrations of cattle supplemented with normal or low concentrations of Zn. Tissue Zn concentrations are well controlled by homeostatic changes in absorption and fecal endogenous excretion of Zn (Underwood and Suttle, 1999). Therefore, if differences in Zn absorption occurred between Zn sources, these would probably not be reflected in differences in plasma and tissue Zn concentrations when Zn was supplemented at only 20 mg/kg diet, due to greater Zn absorption resulting in greater fecal endogenous Zn excretion. However, consistent with previous studies (Rojas et al., 1995; Kincaid et al., 1997; Cao et al., 2000), supplementation with high concentrations (500 mg/kg) of Zn in the present study resulted in elevated concentrations of Zn in plasma, liver, kidney, and bone shaft.

Performance also was similar for calves supplemented with ZnSO₄ and ZnProt in the present study. In contrast, steers fed a high concentrate diet supplemented with 25 mg of Zn/kg from ZnProt tended to gain faster and more efficiently than steers supplemented with ZnO or unsupplemented controls (Spears and Kegley, 2002). However, the addition of 10 mg of Zn/kg from ZnProt or a Zn polysaccharide complex did not improve performance of calves fed a corn and grass silage-based diet relative to controls and ZnO-supplemented calves (Kessler et al.,

2003). Supplementation of growing cattle with Zn methionine has improved (Spears, 1989) or had no effect on gain and gain:feed (Greene et al., 1988) compared with cattle supplemented with inorganic Zn.

When Zn was supplemented at 500 mg/kg diet for 14 d, calves receiving ZnProt had greater Zn concentrations in duodenal homogenates, plasma, liver, and kidney than calves fed ZnSO₄. These results are consistent with ZnProt being absorbed more efficiently than ZnSO₄ when supplemented at high concentrations. Increased duodenal uptake of Zn from ZnProt could be explained by ZnProt interacting less than ZnSO₄, with antagonists that form insoluble complexes. Alternatively, Zn from ZnProt may have been associated with ligands that facilitated Zn uptake in the duodenum. Ashmead et al. (1985) suggested that metal ions may be absorbed as part of a metal:peptide complex, thereby facilitating absorption of Zn via intestinal transport mechanisms distinct from inorganic Zn.

Plasma and kidney Zn concentrations were greater for both the HiZnProt and HiZnM treatments compared with the HiZnSO₄ treatment. However, liver Zn concentrations only differed among calves in the HiZnProt and HiZnSO₄ treatments. Results obtained in the present study agree with previous studies (Rojas et al., 1995; Kincaid et al., 1997; Cao et al., 2000), where ruminants supplemented with organic Zn had greater plasma and/or tissue Zn concentrations than those fed inorganic Zn when Zn was supplemented at relatively high concentrations. Cao et al. (2000) reported greater Zn concentrations in liver, kidney, and pancreas of lambs supplemented with 1400 mg of Zn/kg for 21 d as ZnProt than in those fed ZnSO₄. Calves supplemented with 300 mg of Zn/kg from a mixture of Zn methionine and Zn lysine had greater serum and liver Zn concentrations than calves fed a similar amount of Zn from ZnO (Kincaid et al., 1997). Lambs supplemented with 360 mg of Zn/kg from Zn lysine had much greater Zn concentrations in kidney, liver, and pancreas than lambs fed ZnSO₄, ZnO, or Zn methionine (Rojas et al., 1995).

Liver MT was not significantly increased by the addition of 20 mg of Zn/kg to the control diet. However, calves supplemented with 500 mg of Zn/kg had greater liver MT concentrations than those receiving 20 mg of Zn/kg. It is well documented that high dietary concentrations of inorganic Zn induces synthesis of MT in liver (Davis and Cousins, 2000). Liver MT was not affected by Zn source even in calves fed 500 mg of Zn/kg. The greater liver Zn concentrations in calves fed the HiZnProt diet compared with those fed the HiZnSO₄ diet would be expected to result in greater production of liver MT. However, Zn from ZnProt may be metabolized differently by hepatocytes than inorganic Zn resulting in the Zn being bound to intracellular ligands other than MT. In

contrast to results obtained in the present study, lambs supplemented with 1400 mg of Zn/kg from ZnProt had greater liver MT than lambs receiving ZnSO₄ (Cao et al., 2000).

Supplementing cattle diets with ZnProt has improved hoof elasticity (Reiling et al., 1992) and overall hoof quality scores (Kessler et al., 2003) compared with inorganic Zn. Results of the present study indicate that improved hoof measurements observed in cattle fed ZnProt are not due to greater hoof Zn concentrations. Hoof Zn concentrations also were similar in cattle supplemented with ZnO or ZnProt for 284 d (Kessler et al., 2003). Smith et al. (1999) observed no differences in hoof Zn concentrations in lactating Holstein cows supplemented with ZnSO₄ or Zn methionine.

CONCLUSIONS

Plasma and tissue Zn concentrations were generally similar in calves receiving ZnSO₄, ZnProt, or a 50:50 mixture of ZnSO₄ and ZnProt, when Zn was supplemented at 20 mg/kg to a basal diet containing 28 mg of Zn/kg. Zinc addition at 20 mg/kg to the basal diet also did not greatly affect plasma and tissue Zn concentrations. Supplementation of 500 mg of Zn/kg for 14 d increased duodenal, liver, kidney, and plasma Zn concentrations compared with the 20 mg of Zn/kg treatments. When Zn was supplemented at 500 mg/kg, calves receiving ZnProt had greater duodenal, liver, kidney, and plasma Zn concentrations than calves receiving ZnSO₄. This suggests greater absorption and/or retention of Zn from ZnProt compared with ZnSO₄ at the high Zn concentration. At the low level (20 mg/kg) of Zn supplementation, normal homeostatic control mechanisms for Zn may have prevented differences in plasma or tissue Zn concentrations due to Zn source and level.

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