Influence of dietary zinc concentration and supplemental zinc source on nutrient digestibility, zinc absorption, and retention in sheep¹

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ABSTRACT: The objective of this study was to assess whether supplemental Zn source or concentration would affect ruminant Zn retention and nutrient digestibility. Thirty-six weaned crossbred Polypay wethers, were sorted by BW to 3 periods and stagger started on a common diet (22 mg Zn/kg DM) for a 52-d depletion period. Day 52 BW was used to assign Zn treatments (3 lambs/treatment/period): no supplemental Zn (CON), or supplemental Zn (40 mg Zn/d) from Zn sulfate (ING; Zinc Nacional, Monterrey, SA, Mexico), Zn methionine (ORG; Zinpro 120; Zinpro, Eden Prairie, MN), or Zn hydroxychloride (HYD; IntelliBond Z; Micronutrients USA LLC, Indianapolis, IN). On day 53 (day 1 of Zn treatments), lambs were moved to metabolism crates for 10 d of adaptation and 5 d of total fecal and urine collection. Blood for plasma Zn analysis was collected on day 52 and day 68. Data were analyzed as a randomized complete design with fixed effects of treatment, period and the interaction, which was significant ($P \geq 0.19$) for day 68 plasma Zn but was removed for all other variables. Contrast statements were used to separate treatment means: CON vs. ZINC (ING, ORG, HYD), ING vs. HYD, and ORG vs. HYD. Day 52 plasma Zn concentrations were similar when CON was compared with ZINC ($P = 0.84$),

and when ING and ORG were compared with HYD ($P \geq 0.19$). Dry matter and neutral detergent fiber digestibility were lesser in ORG compared with HYD ($P = 0.05$) and organic matter and acid detergent fiber digestibility tended ($P \leq 0.08$) to be lesser in ORG compared with HYD. Intake and fecal excretion of Zn was lesser, while apparent absorption of Zn was greater, in CON compared with ZINC ($P \leq 0.001$). Zinc retained as a percent of Zn intake was greater in CON compared with ZINC ($P = 0.001$). Zinc retained (mg/d) was similar in CON compared with ZINC ($P = 0.58$) and when ING or ORG were compared with HYD ($P \geq 0.83$). There was a treatment $\times$ period interaction for day 68 plasma Zn where treatments did not differ for periods 1 and 3 but ORG lambs had increased plasma Zn in period 2 compared with other treatments ($P = 0.02$). Lambs receiving no supplemental Zn had increased apparent absorption, suggesting Zn absorption may be upregulated in these lambs. Similarities in Zn retention across treatments suggests Zn requirements of these lambs were met regardless of supplementation concentration or source. Nutrient digestibility was improved in HYD lambs compared with ORG, and further work is needed to clarify the influence of supplemental Zn source on nutrient digestion.

Key words: nutrient digestibility, sheep, trace mineral source, zinc metabolism

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INTRODUCTION

Trace minerals (TM) are essential in livestock diets and support optimum animal health

(Mertz, 1981). While Zn is involved in a vast assortment of physiological processes, lack of reliable biomarkers makes evaluating bioavailability of Zn sources difficult. A commonly used method to compare Zn sources is to calculate Zn retention by animals (Spears, 1989; Spears et al., 2004; Buff et al., 2005; Shaeffer et al., 2017). Shaeffer et al. (2017) reported increased Zn retention and absorption in steers supplemented with Zn from Zn hydroxychloride compared with steers supplemented with Zn sulfate (ZnSO_4). Supplementation with an organic Zn source increased Zn retention in lambs compared with inorganic Zn supplementation (Spears, 1989).

Nutrient digestibility may also be influenced by TM source. Lambs supplemented with Zn from Zn methionine had increased ADF digestibility (Garg et al., 2008), and dairy cows receiving supplemental Cu, Mn, and Zn from hydroxy sources had increased NDF digestibility compared with those supplemented with sulfate sources (Faulkner and Weiss, 2017). While there are a variety of Zn sources that can be utilized in ruminant diets, it can be difficult to make comparisons between the sources; thus, the objectives of this study were to determine the effect of Zn source (Zn hydroxychloride, Zn methionine, and ZnSO_4) on Zn retention and nutrient digestibility. It was hypothesized that lambs receiving Zn hydroxychloride would have similar Zn retention and nutrient digestibility compared with those receiving Zn methionine and increased Zn retention and nutrient digestibility compared with those receiving ZnSO_4 .

MATERIALS AND METHODS

All experimental procedures and protocols were approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC: log number 7-16-8319-O).

Thirty-six weaned crossbred Polypay wethers were selected from the flock at the Iowa State University Sheep Teaching Farm (Ames, Iowa) and BW was measured (average BW 31.5 ± 3.56 kg). A date range for lambing, but no specific birth-date information for each lamb was recorded in this flock, and it was estimated that lambs averaged 2.5 mo of age on the day of selection. The selected lambs were sorted by BW into 3 periods, and were enrolled 3 wk apart into a 52-d Zn depletion period. Upon starting the 52-d depletion period, lambs were fed a common total mixed ration (TMR; Table 1) that would be fed throughout the trial. Lambs were staggered started in order to accommodate space in the Iowa State University

Table 1. Ingredient and nutrient composition of common total mixed ration (TMR)¹

Ingredient	% of Diet DM
Cracked corn	39.00
Cottonseed hulls	20.00
Corn gluten meal	14.00
Grass hay	13.00
Micronutrients and carrier ^{2,3}	10.00
Molasses	4.00
Analyzed composition ⁴	
DM	88.00
OM	84.52
NDF	36.12
ADF	21.30
CP ⁵	19.59
EE ⁵	2.85
Trace minerals, mg/kg DM	
Zn	22
Cu	5.4
Fe	111
Mn	32.7

¹Common TMR fed to all lambs during depletion period. During metabolism period, CON lambs continued on this diet while ING lambs received the common TMR plus 40 mg/d of Zinc (Zn) from ZnSO_4 , ORG lambs received common TMR plus 40 mg/d of Zn from Zn methionine, and HYD lambs received common TMR plus 40 mg/d Zn from Zn hydroxychloride.

²Provided per kg of diet DM: 0.2 mg Co (as cobalt carbonate), 25 mg Mn (as manganese sulfate), 0.22 mg Se (as sodium selenite), 0.6 mg I (as calcium iodate).

³Provided as percent of diet DM: 5.92% ground corn, 2.1% calcium propionate, 0.25% dicalcium phosphate, 0.5% urea, 0.1% vitamin ADE premix (contained 900,000 IU of vitamin A, 225,000 IU of vitamin D, and 180 IU of vitamin E per kilogram of premix), 0.015% Bovatec (Provided lasalocid at 27 mg/908 kg diet DM; Zoetis, New York, NY), 0.5% salt, 0.1% Mg oxide, 0.5% ammonium chloride.

⁴EE = ether extract.

⁵Analysis performed by Dairyland Laboratories (Arcadia, WI).

metabolism facility (Ames, Iowa). When period 1 began, the 52-d depletion period lambs in periods 2 and 3 remained on a weaning ration until their respective depletion period start date. During the depletion period, lambs were housed at the Iowa State University Sheep Teaching Farm. Lamb BW at the start of depletion was similar across periods (30.9, 32.0, and 31.5 kg for periods 1, 2, and 3, respectively). All lambs in each of the 3 periods consumed the common TMR for a total of 52 d and were weighed weekly. Feed was offered at 4% of BW on a dry matter (DM) basis, adjusted weekly based on lamb BW, and all lambs had ad libitum access to water.

On day 52 of each period, lambs were blocked by BW within group to be assigned to 1 of 4 dietary treatments for the metabolism period (3 lambs

per treatment per group). On day 52 of each period, lambs were transported 6 km from the sheep teaching farm to the metabolism facility and were acclimated to the facility for 24 h in a comingled pen. During the 24 h acclimation period, lambs were offered the common TMR at the same rate as the previous day and given ad libitum access to water. On day 1 of the metabolism period, sheep were treated for internal parasites with 10 mL of Cydectin oral drench (Boehringer Ingelheim, St. Joseph, MO), and moved to individual metabolism crates [123.2 cm (length) by 41.9 cm (width) by 93.4 cm (height)]. Metabolism crates were designed such that 2 lambs were housed next to each other and could see one another but were not allowed physical contact. The steel metabolism crates were painted with a Zn-free paint (Sherwin-Williams Co, Inc., Cleveland, OH) prior to the start of the trial, and the floors were covered with a plastic coating to limit Zn contamination. Lambs were fed out of stainless steel feeders and water was provided in plastic waterers. Lambs began dietary treatments on day 1 of the metabolism period which consisted of: 1) Control (**CON**)—common TMR, 2) Inorganic (**ING**)—CON supplemented with 40 mg of Zn from ZnSO_4 (Zinc Nacional, Monterrey, SA, Mexico), 3) Organic (**ORG**)—CON supplemented with 40 mg of Zn from Zn methionine (Zinpro 120; Zinpro, Eden Prairie, MN), or 4) Hydroxy (**HYD**)—CON supplemented with 40 mg of Zn from Zn hydroxychloride (IntelliBond Z; Micronutrients USA LLC, Indianapolis, IN). All Zn treatments were delivered to lambs daily at 0700 h as a top dress with 100 g of fine ground corn, along with 228 g of the common TMR (as fed basis) to ensure consumption of supplemental Zn. Control lambs were offered a top dress containing 100 g of fine ground corn and 228 g of the common TMR on an as fed basis.

Lambs were allowed to acclimate to the metabolism crates for 10 d during which feed was offered at 105% of the previous day's intake. Adaptation was followed by 5 d of total fecal and urine collection. During the collection period, lambs were limit fed at 95% of their day 10 adaptation feed intake to limit orts during collection, and water was offered ad libitum.

Sample Collection and Analytical Procedures

Prior to the beginning of the trial, a water sample was collected from the automatic waterers located at the Iowa State University Sheep Teaching Farm and analyzed to contain 0.03 mg Zn/L. Water samples collected from the metabolism facility contained 0.04, 0.03, and 0.03 mg Zn/L in periods 1,

2, and 3, respectively. Individual lamb water intake was not measured.

During the 5-d total fecal and urine collection, TMR, feed refusals, feces, and urine were collected, composited, and dried as described by [Lundy et al. \(2015\)](#) and [Carmichael et al. \(2018\)](#). Briefly, urine samples were collected in plastic containers that had been acid washed with 10% hydrochloric acid prior to the start of the trial. Each urine collection container was covered with a layer of polyester mesh to limit contamination from feces and feed. Fecal collection containers were lined with plastic liners replaced daily and fecal samples were dried in plastic acid-washed containers. Dried feed refusals, feces, and TMR samples were ground to fit through a 2-mm screen in a Retsch ZM 100 grinding mill (Retsch GmbH, Haan, Germany). Dry matter intake and OM intake, as well as DM and OM digestibility were determined as described by [Pogge et al. \(2014\)](#). Neutral detergent fiber and ADF analysis was performed on feeds, feces, and feed refusals in duplicate using an ANKOM 200 fiber analyzer (Ankom Technology, Macedon, NY) using the methods of [Van Soest et al. \(1991\)](#). Alpha-amylase was utilized, but sulfite was excluded from the NDF procedure. A brome grass hay sample was included in each run to verify consistency across runs (interassay CV of 1.7% and 4.2% for NDF and ADF, respectively). Total mixed ration composites from each period were analyzed via wet chemistry for crude protein (method 990.03; [AOAC, 1996](#)) and ether extract (method 920.39; [AOAC, 1996](#)) by Dairyland Laboratories (Arcadia, WI).

Blood was collected for each group at the end of the depletion period (day 52) and at the end of the 5-d collection period for determination of plasma Zn concentrations. Blood was collected by jugular venipuncture into 7 mL trace element K_2 EDTA blood collection tubes (Becton Dickinson and Company, Franklin Lakes, NJ), placed on ice and transported to the laboratory. Blood was centrifuged at $1,200 \times g$ for 10 min at 4 °C, and plasma was stored at -80 °C until further analysis. Feces, TMR, feed refusals, and plasma were analyzed for Zn content via inductively coupled plasma atomic emission spectrometry (Optima 7000 DV, PerkinElmer, Waltham, MA) as described by [Pogge et al. \(2014\)](#). Prior to analysis, TMR, fecal, and feed refusals were acid digested (CEMS MARSXpress, Matthews, NC) with TM grade nitric acid and diluted to 20% nitric acid with deionized water. Urine and plasma samples were prepared for Zn analysis as previously described by [Pogge and Hansen \(2013\)](#) and [Pogge et al. \(2014\)](#). Total mineral content of TMR, feed

refusals, feces, and urine as well as daily mineral intake and output, mineral retention, and apparent absorption were calculated as previously described by Pogge et al. (2014).

Statistical Analysis

Data were analyzed by ANOVA as a randomized complete design using the Mixed procedure of SAS (SAS version 9.4, SAS Inst. Inc., Cary, NC). The initial model for analysis of all data included the fixed effects of treatment and period and the interaction. Contrast statements were used to compare treatment means and consisted of CON vs. ZINC (ING, ORG, and HYD), ING vs. HYD, and ORG vs. HYD. All data were examined for outliers using Cook's D statistics, and no outliers were identified. Significance was declared at $P \leq 0.05$ and tendencies were declared at $0.06 \leq P \leq 0.10$. The interaction of treatment and period was only significant for final plasma Zn data, and the interaction was removed from the model for all other variables ($P \geq 0.19$).

RESULTS

Depletion Period Performance

Body weight at the end of the depletion period, which was used to block lambs to metabolism

period treatments, was similar when CON (43.3 kg) was compared with ZINC ($P = 0.91$) and when ING (43.1 kg) or ORG (43.1 kg) lambs were compared with HYD (43.2 kg; $P \geq 0.92$). Lamb BW at end of the depletion (start of metabolism) tended to be affected by period ($P = 0.06$) where period 3 (41.3 kg) lambs were lighter than period 1 (44.2) and period 2 (44.0) lambs. Average daily gain (ADG) was affected by period ($P < 0.01$) where period 3 (0.19 kg/d) lambs had decreased ADG during the depletion period compared with period 1 (0.26 kg/d) and period 2 (0.23 kg/d) lambs.

Nutrient Intake, Output, and Digestibility by Lambs

Nutrient intake, output, and digestibility data for the 5-d collection period are reported in Table 2. No differences were observed in intake and output for DM, OM, NDF, and ADF or urine production when analyzed through individual contrasts ($P \geq 0.21$). Dry matter, OM, NDF, and ADF digestibilities were similar between CON and ZINC lambs ($P \geq 0.42$) and ING and HYD lambs ($P \geq 0.29$). Dry matter and NDF digestibilities were greater in HYD lambs compared with ORG ($P < 0.05$). There was a tendency for OM and ADF digestibilities to be greater in HYD lambs compared with ORG ($P \leq 0.08$).

Intake of DM, OM, NDF, and ADF were greatest in period 1 ($P \leq 0.04$). A period effect was

Table 2. Influence of dietary Zn on nutrient intake, output, and digestibility by lambs

	Treatment ¹				SEM	Contrast statements			
	CON	ING	ORG	HYD		CON vs. ZINC	ING vs. HYD	ORG vs. HYD	Prd P -value ³
Lambs, (n)	9	9	9	9					
Daily nutrient intake									
DM, kg	1.21	1.23	1.19	1.18	0.043	0.92	0.41	0.94	0.04
OM, kg	1.16	1.19	1.15	1.14	0.041	0.90	0.39	0.92	0.02
NDF, kg	0.41	0.43	0.41	0.40	0.016	0.93	0.27	0.83	0.003
ADF, kg	0.24	0.25	0.24	0.23	0.009	0.97	0.28	0.62	0.001
Daily nutrient output									
Fecal DM, kg	0.34	0.36	0.35	0.33	0.016	0.75	0.23	0.32	0.31
Fecal OM, kg	0.32	0.33	0.33	0.31	0.015	0.83	0.28	0.40	0.29
Fecal NDF, kg	0.23	0.24	0.24	0.22	0.012	0.98	0.21	0.25	0.39
Fecal ADF, kg	0.15	0.16	0.16	0.15	0.008	0.69	0.22	0.23	0.38
Urine, L	1.07	0.95	1.00	1.05	0.101	0.58	0.49	0.76	0.003
Nutrient digestibility									
DM, %	71.6	71.0	70.1	72.0	0.651	0.42	0.29	0.05	0.03
OM, %	72.6	72.1	71.3	72.8	0.622	0.49	0.39	0.08	0.03
NDF, %	43.1	43.3	40.6	45.0	1.53	0.93	0.43	0.05	0.001
ADF, %	36.1	35.1	32.7	36.9	1.59	0.50	0.42	0.07	0.001

¹CON: common TMR with no supplemental zinc, ING: common TMR plus 40 mg/d of Zinc (Zn) from ZnSO₄, ORG: common TMR plus 40 mg/d of Zn from Zn methionine, HYD: common TMR plus 40 mg/d of Zn from Zn hydroxychloride.

²Contrast is CON vs. the average of all 3 Zn-supplemented treatments.

³Prd: period; no Treatment $\times$ Prd interactions ($P \geq 0.38$).

noted for daily urinary production where period 3 lambs had lesser urinary production than lambs in period 1 or 2 ($P = 0.003$). Diet digestibility of DM, OM, and ADF were least in period 3 lambs ($P \leq 0.03$). Digestibility of NDF differed ($P < 0.001$) across periods where period 1 had the greatest NDF digestibility, period 2 was intermediate, whereas NDF digestibility by period 3 lambs was least.

Daily Zn Intake, Excretion, Absorption, and Retention

Daily Zn intake and excretion, Zn apparent absorption and retention, and plasma Zn data are reported in Table 3. Daily Zn intake and fecal excretion were lesser in CON lambs compared with ZINC ($P < 0.001$). Zinc intake and fecal excretion were not different in ING or ORG lambs compared with HYD ($P \geq 0.32$). Urinary excretion of Zn did not differ in CON vs. ZINC, and ING or ORG lambs compared with HYD ($P \geq 0.38$). Zinc apparent absorption and retention as a percent of intake was greater in CON compared with ZINC ($P \leq 0.001$). When ING or ORG lambs were compared with HYD lambs, Zn apparent absorption and retention as a percentage of intake was not different ($P \geq 0.76$). Zinc retained (mg/d) was similar in CON

vs. ZINC, as well as ING or ORG lambs compared with HYD ($P \geq 0.58$). Period influenced Zn intake and fecal Zn excretion ($P < 0.001$), where Zn intake was greatest in period 1 (62.4 mg/d), and similar in periods 2 (53.8 mg/d) and 3 (54.9 mg/d). Fecal Zn excretion was greatest in period 1 lambs (56.0 mg/d) and least in period 2 lambs (44.9 mg/d), and intermediate in period 3 lambs (49.3 mg/d). Urinary Zn excretion was greater ($P = 0.009$) in period 1 lambs (0.38 mg/d) vs. period 3 lambs (0.22 mg/d), while period 2 lambs (0.31 mg/d) were intermediate.

Plasma Zn measured on day 52 at the end of the depletion period, immediately prior to onset of Zn supplementation, was similar in CON vs. ZINC ($P = 0.84$), and when ING or ORG lambs were compared with HYD ($P \geq 0.19$). Final plasma Zn concentrations measured at the end of the metabolism period (day 68 of study, 15 d of Zn supplementation) displayed an interaction between treatment and period ($P = 0.02$), where, within period, treatments did not differ for periods 1 and 3, but the ORG treatment was greater than other treatments in period 2 ($P < 0.01$).

DISCUSSION

The essentiality of Zn in livestock diets has been well established (Mertz, 1981). However, a

Table 3. Influence of supplemental Zn source and concentration on plasma Zn, Zn intake, fecal and urine excretion, Zn apparent absorption, and Zn retention by lambs

	Treatment ¹				SEM	Contrast statements ²			P-values ³	
	CON	ING	ORG	HYD		CON vs. ZINC	ING vs. HYD	ORG vs. HYD	Prd	Trt × Prd
Lambs, (n)	9	9	9	9						
Zn										
Day 52 plasma Zn ⁴ , mg/L	0.93	0.97	0.86	0.93	0.037	0.84	0.43	0.19	0.21	0.90
Day 68 plasma Zn ⁵ , mg/L	1.04	0.96	1.07	0.92	0.065	0.47	0.65	0.12	-	0.02
Intake, mg/d	28.0	68.3	65.3	66.5	1.25	0.001	0.32	0.51	0.001	-
Fecal excretion, mg/d	21.8	61.3	58.1	59.0	1.62	0.001	0.32	0.71	0.001	-
Urinary excretion, mg/d	0.27	0.29	0.32	0.33	0.04	0.38	0.54	0.92	0.009	-
Apparent absorption, %	22.7	10.3	10.9	11.4	2.53	0.001	0.76	0.88	0.09	-
Retained, mg/d	5.9	6.7	6.9	7.2	1.55	0.58	0.83	0.89	0.23	-
Retained, % of intake	21.8	9.9	10.3	10.9	2.55	0.001	0.77	0.88	0.09	-

¹CON: common TMR with no supplemental zinc, ING: common TMR plus 40 mg/d of Zinc (Zn) from ZnSO₄, ORG: common TMR plus 40 mg/d of Zn from Zn methionine, HYD: common TMR plus 40 mg/d of Zn from Zn hydroxychloride.

²Contrast is CON vs. the average of Zn-supplemented treatments.

³Trt: treatment Prd: period.

⁴Initial plasma Zn concentrations measured on d 52 of the study prior to moving to the metabolism facility.

⁵Final plasma Zn concentrations measured on d 68 of the study upon removal of lambs from metabolism crates. Trt × Prd interaction indicates that within period, treatments did not differ for period 1 and 3, but the ORG treatment was greater than other treatments in period 2 (CON: 0.95 mg/L, ING 0.84 mg/L, ORG: 1.40 mg/L, HYD: 0.93 mg/L; $P < 0.01$).

lack of reliable and consistent biomarkers makes evaluating the bioavailability of Zn sources for livestock difficult. Even when Zn status is marginal to deficient, plasma Zn concentrations remain under tight homeostatic control, making them an unreliable biomarker (Hambidge, 2003). Calculation of Zn retention may serve as a better method of comparing Zn sources. This research was conducted to evaluate Zn retention and apparent absorption as a proxy for bioavailability in lambs fed a marginally Zn-deficient diet with either no supplemental Zn or supplemental Zn as Zn hydroxychloride, Zn methionine, or ZnSO₄.

Zinc supplementation has been shown to affect Zn absorption, and previous work has shown that when dietary Zn increases, absorption of Zn decreases (Weigend and Kirchgessner, 1980; Sandström and Cederblad, 1980). In the present study, all lambs received a TMR containing 22 mg Zn/kg DM for 52 d prior to beginning repletion diets while ZINC lambs received an additional 40 mg supplemental Zn/d. The small ruminant NRC (2007) suggests that lambs used in the present study (40 kg; ADG 250 g/d) require 45 mg Zn/d; therefore, CON lambs received 62% of the dietary Zn requirement for lambs of their size, whereas ZINC lambs received 148% of the dietary Zn requirement. Control lambs demonstrated Zn apparent absorption rates twice that of the lambs supplemented with Zn. This may suggest the CON lambs were at least marginally Zn deficient, as Zn-depleted rats demonstrate increased Zn absorption due to increased kinetics of Zn uptake (Hoadley et al., 1987).

Apparent absorption of Zn has been shown by others to be approximately 20% in sheep (Spears, 1989), steers (Nockels et al., 1993; Shaeffer et al., 2017), and lactating dairy cows (Faulkner et al., 2017). While Shaeffer et al. (2017) implemented a 14-d depletion period, lambs in the present study underwent a 52-d depletion period and exhibited average apparent absorption rates of 10.8% for Zn-supplemented lambs, lesser than that reported by others. This suggests that length and severity of depletion likely impact Zn absorption mechanisms upon repletion of Zn. Zinc absorption may have been downregulated in Zn-supplemented lambs after realimentation of Zn at the start of the metabolism period. Though intestinal expression of transporters was not assessed in the present study, low dietary Zn leads to increased expression of the Zn transporter ZIP4 in the epithelial cells of the small intestine (Liuzzi et al., 2004). Thus, the marginally Zn-deficient CON lambs likely had greater ZIP4

expression, while ZINC lambs may have quickly downregulated expression of ZIP4 at the onset of Zn supplementation following the prolonged period of moderate Zn depletion. Similar to the results in the present study, Faulkner et al. (2017) reported no difference in Zn apparent absorption in dairy cows supplemented with Zn from ZnSO₄ or Zn hydroxychloride.

Dietary antagonisms may affect the bioavailability of Zn sources, as the presence of dietary antagonists such as phytate and Ca have been shown to decrease Zn absorption in monogastrics (Mills, 1985). While microbial phytase can break down phytate in the rumen, some phytate can appear in the small intestine and alter Zn absorption (Clark et al., 1986). In the present study, the basal diet was formulated to meet dietary P and Ca requirements, without providing excess. The improved bioavailability of Zn hydroxychloride compared with ZnSO₄ observed by Shaeffer et al. (2017) may have in part been due to Zn hydroxychloride being better able to overcome dietary antagonism from high Ca concentrations in the basal diet. Further work is needed to clarify the effectiveness of various Zn sources to most efficiently provide Zn to the ruminant when different dietary antagonists such as Ca, S, or Fe are present.

It was hypothesized that lambs supplemented with Zn hydroxychloride would have similar Zn retention compared with those supplemented with Zn methionine, and increased Zn retention compared with those supplemented with ZnSO₄. However, in the present study, daily Zn retention was similar among treatments, suggesting the Zn requirements of these lambs were approximately 6–7 mg/d. Similar Zn retention values were observed across treatments, suggesting the Zn requirements of these lambs were met regardless of supplemental Zn source. Faulkner et al. (2017) also observed no effect of supplemental Zn source on Zn retention, but that dietary fiber type did effect Zn retention. Calculated from the data provided by Faulkner et al. (2017), it is interesting to note that Zn retained as a percentage of intake was about 5 percentage units greater in cows fed a by-product-based diet (19.6%) compared with a forage-based diet (14.4%). This may allude to the importance of considering interactions between Zn and dietary nutrients when comparing supplemental Zn sources.

Improvements, or trends for improvements, in DM, OM, NDF, and ADF digestibilities in HYD-supplemented lambs compared with ORG lambs were noted in the present study. Previous work has shown alterations in DM digestibility due to

Zn supplementation and source; however, many of these changes were observed in experimental conditions utilizing potentially pharmacological concentrations of Zn chloride (Arelovich et al., 2000) and ZnSO₄ (Bonhomme et al., 1979; Eryavuz and Dehority, 2009). Eryavuz and Dehority (2009) hypothesized that lesser cellulose digestion may be contributing to in vitro DM disappearance differences through inhibition of cellulolytic enzymes rather than a toxic effect of Zn on rumen microbes. Solubility of Zn sources in the gastrointestinal tract can be variable with Zn chloride being highly soluble in water (Windholz et al., 1976), and ZnSO₄ being highly soluble in both neutral and acidic conditions (Sandoval et al., 1997). Hydroxy TM have low water solubility but are soluble in acidic conditions (Cao et al., 2000), and organic TM including Zn methionine may remain intact in rumen conditions (Spears 1996). Studies evaluating the solubility of Zn sources have been conducted in vitro, and solubility of Zn sources in the present trial may have been affected by the rumen environment.

Others have evaluated nutrient disappearance and digestibility utilizing Zn concentrations that are more similar to the Zn requirement of beef cattle (NASEM, 2016) or industry-relevant supplementation practices (Samuelson et al., 2016). Genther and Hansen (2015) noted lesser in situ DM disappearance from steers supplemented with sulfate-bound TM (Zn, Mn, and Cu) compared with non-TM-supplemented steers and those supplemented with hydroxy TM. Neutral detergent fiber disappearance in the rumen using in situ techniques was similar regardless of TM supplementation strategy, and the authors suggested digestion of nutrients other than NDF may be affected by TM supplementation. However, supplementation of hydroxy TM (Zn, Mn, and Cu) has been shown to increase total tract NDF digestibility in lactating dairy cows compared with those supplemented with sulfate sources (Faulkner and Weiss, 2017). In contrast, in the current study, total tract nutrient digestibility, including NDF digestibility, was similar between ING and HYD lambs. Similarities in both Zn supplementation and total diet NDF were noted between Faulkner and Weiss (2017; 35 mg Zn/kg DM; 36.4% NDF) and the present study (33 mg Zn/kg DM; 36.1% NDF). However, the by-product diet fed in Faulkner and Weiss (2017) contained highly digestible fiber sources including dried corn gluten feed, dried beet pulp and soy hulls, while fiber was largely provided from cottonseed hulls and grass hay in the present study. This may suggest that rate of ruminal fiber fermentation and overall

fiber characterization are important factors when assessing influence of supplemental TM source on fiber digestibility.

Although Genther and Hansen (2015) and Faulkner and Weiss (2017) compared hydroxy or sulfate sources of several TM (Zn, Mn, and Cu), the present study focused solely on Zn. The trends for differences in fiber digestion in the present study among sources suggest that Zn source may influence fiber digestion in ruminants; however, further work is needed to elucidate factors other than Zn source that might be impacting fiber digestibility, including fiber source. The unexpected improvement in nutrient digestibility in HYD compared with ORG lambs in the present study may suggest that these 2 sources interact differently within the ruminant gastrointestinal tract. This finding warrants further research to determine TM supplementation strategies to optimize rumen microbial populations in concert with nutrient digestibility and animal growth.

Period effects were noted for Zn metabolism and nutrient digestibility variables, and may have been due to stagger starting lambs on the Zn depletion diet. All lambs were weighed on a single day prior to the start of the trial, with the heaviest lambs assigned to period 1, middle lambs to period 2, and lightest lambs to period 3. Lambs were sourced from a single flock that did not record birthdate information. Thus, it is possible that the lighter lambs in periods 2 and 3 were younger than those in period 1, or some lambs in periods 2 and 3 may have simply grown slower than those in period 1. Although period effects were noted for variables throughout this trial, it is important to note that a treatment × period effect was only noted for day 68 plasma Zn concentrations.

Plasma Zn concentrations at the end of depletion and after 15 d of Zn supplementation were largely unaffected by dietary Zn concentration. This may be due to the inability of plasma Zn to reflect subtle changes in Zn status (Hambidge, 2003). It is possible that a longer duration of Zn supplementation would have resulted in differences in plasma Zn concentrations, though this appears to vary across the literature. Relative to ZnSO₄ steers supplemented with Zn hydroxychloride had greater plasma Zn concentrations after 40 d of Zn supplementation, while Zn absorption and retention was increased during a 5-d collection period from day 8 through 12 of supplementation (Shaeffer et al., 2017). However, blood samples were not analyzed during the collection period in that trial. Spears et al. (2004) noted that dietary Zn and Zn source

affected plasma Zn concentrations after 42 d of Zn supplementation while absorption and retention of Zn remained similar across treatments when determined from day 14 through 19 of the Zn supplementation period. Furthermore, improvements in efficiency and performance in steers have been noted with different sources of supplemental Zn while plasma Zn remained unaffected when measured after 84 d and 196 d of Zn supplementation (Spears and Kegley, 2002). These findings add to the growing collection of data that suggest plasma Zn is an inconsistent and unreliable biomarker for determination of Zn status and bioavailability.

In conclusion, Zn retention (mg/d) was similar in all lambs regardless of supplemental Zn source or dietary Zn concentration. Apparent absorption of Zn was similar between Zn-supplemented treatments, and lesser than that of CON lambs. Future research is needed to identify mechanisms affecting apparent absorption in Zn-supplemented lambs, specifically evaluation of intestinal Zn transporter abundance in ruminants supplemented with different Zn sources. Similar results across treatments for Zn retention (mg/d) suggests that the Zn requirement for these lambs was met by the diets regardless of source, and potential alterations in absorptive mechanisms may be masking differences in Zn retention due to source. The results of the current study suggest that nutrient digestibility maybe influenced by supplemental Zn source as nutrient digestibilities were improved in HYD compared with ORG lambs. Zinc is robustly incorporated into enzymes throughout the body and is critical for most metabolic processes. Further research is needed to elucidate dietary factors influencing Zn metabolism in order to refine Zn supplementation strategies in ruminants.

Conflict of interest statement. None declared.

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