Effects of supplemental zinc and manganese on ruminal fermentation, forage intake, and digestion by cattle fed prairie hay and urea

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Effects of supplemental zinc and manganese on ruminal fermentation, forage intake, and digestion by cattle fed prairie hay and urea[1,2]

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ABSTRACT: One in vitro and one in vivo metabolism experiment were conducted to examine the effects of supplemental Zn on ruminal parameters, digestion, and DMI by heifers fed low-quality prairie hay supplemented with urea. In Exp. 1, prairie hay was incubated in vitro for 24 h with five different concentrations of supplemental Zn (0, 5, 10, 15, and 20 ppm) and two concentrations of supplemental Mn (0 and 100 ppm), both provided as chloride salts. Added Mn increased ($P < 0.02$) IVDMD, but added Zn linearly decreased ($P < 0.03$) IVDMD. Added Zn tended to increase the amount of residual urea linearly ($P < 0.06$) at 120 min and quadratically ($P < 0.02$) at 180 min of incubation, although added Mn counteracted these effects of added Zn. Six 363-kg heifers in two simultaneous 3 × 3 Latin squares were fed prairie hay and dosed once daily via ruminal cannulas with urea (45 or 90 g/d) and with Zn chloride to provide the equivalent of an additional 30 (the dietary requirement), 250, or 470 ppm of dietary Zn. After a 7-d adaptation period, ruminal contents were sampled 2, 4, 6, 12, 18, 21, and 24 h after the supplement was dosed. Supplemental Zn did not alter prairie hay DMI (mean = 4.9 kg/d) or digestibility, although 470 ppm added Zn tended to decrease ($P < 0.06$) intake of digestible DM, primarily due to a trend for reduced digestibility with 470 ppm supplemental Zn. Zinc × time interactions were detected for both pH ($P = 0.06$) and NH_3 ($P = 0.06$). At 2 h after dosing, ruminal pH and ruminal ammonia were linearly decreased ($P < 0.05$; $P < 0.01$) by added Zn. At 5 h after feeding, ruminal pH was linearly increased ($P < 0.05$) by added Zn, suggesting that added Zn delayed ammonia release from urea. The molar proportion of propionate in ruminal fluid was linearly and quadratically increased ($P < 0.02$; $P < 0.01$) whereas the acetate:propionate ratio was linearly and quadratically decreased ($P = 0.02$; $P < 0.05$) by added Zn. Through retarding ammonia release from urea and increasing the proportion of propionate in ruminal VFA, Zn supplementation at a concentration of 250 ppm may decrease the likelihood of urea toxicity and increase energetic efficiency of ruminal fermentation.

Key Words: Cattle, Propionic Acid, Rumen, Urea, Zinc

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Introduction

Urea is widely used because it is an economical source of nonprotein N for ruminants. Inadequate ruminal ammonia can reduce microbial activity (Satter and Slyter, 1974). Supplemental urea usually increases ruminal ammonia concentrations, often increases forage DMI, and may enhance microbial protein synthesis in the rumen (Bowen et al., 1998). However, excesses of ruminal ammonia can decrease N retention, reduce productivity (Doyle, 1987), and prove toxic. Ammonia accumulates in the rumen whenever rate of production exceeds rate of utilization.

Decreasing rate of NH_3 release from urea can prove beneficial 1) to avoid NH_3 spikes and consequent loss from the rumen and 2) to maintain ruminal ammonia at an adequate level for a longer postfeeding time period. Various methods to modulate urea degradation have been developed. These include complexing or coating urea with a variety of compounds such as oil or carbohydrates and treatment with formaldehyde or acids (Forero et al., 1980; Makkar and Negi, 1988; Jinderpal and Kaushal, 1993). Elevated concentrations of certain minerals also may retard ammonia release. Spears and Hatfield (1978) reported that elevated concentrations

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of Cu, Zn, Cd, Sr, Ca, Co, Mn, Ba, and Mg can inhibit ammonia accumulation from urea in vitro. High dietary levels of Zn (600 ppm) or Zn plus Mn (600 + 550 ppm) also have improved urea utilization, N balance, and ADG by sheep (Rodriguez et al., 1995). In a preliminary in vitro trial, combinations of five different Zn and Mn concentrations, ranging from 0 to 20 ppm and 0 to 100 ppm, respectively, were tested. The depression in urea degradation was greater with supplemental Zn than with supplemental Mn (Arelovich, 1998). The objectives of this study were to measure 1) effects of elevated concentrations of Zn and Mn on in vitro disappearance of urea and prairie hay dry matter and 2) the impact of increased intake of Zn on ruminal metabolism, DMI, and digestibility of prairie hay by heifers.

Materials and Methods

Experiment 1

Rates of disappearance of urea and DM in vitro at various Zn and Mn concentrations were appraised by incubating prairie hay with a mixture of ruminal fluid and buffer solution. Martinez and Church (1970) reported that when Zn and Mn concentrations exceeded 20 and 100 ppm, respectively, activity of ruminal bacteria was depressed. Consequently, these concentrations set the maximum levels to be tested in vitro.

Based on results of a preliminary in vitro trial, we tested four concentrations of supplemental Zn (0, 5, 10, 15, and 20 ppm) with two concentrations of Mn (0 and 100 ppm) in vitro. The mineral solutions were prepared from ZnCl and tetrathydrated MnCl.

In Vitro Procedure. Our in vitro procedure was altered slightly from that of Tilley and Terry (1963). Three 540-kg cannulated steers were fed 7.5 kg prairie hay plus 500 g of a supplement (89.5% ground corn, 10% urea, and 0.5% NaCl) at 0900 each day for 15 d prior to the first collection of ruminal fluid for IVDMD and ureolysis measurements. The entire in vitro procedure was replicated three times; ureolysis was quantified in all three runs, whereas IVDMD and ureolysis were measured only in the final two in vitro runs. Ruminal fluid was obtained from each steer 24 h after the last meal. The fluid from all three steers was mixed, filtered through four layers of cheesecloth twice, mixed 1:1 (vol:vol) with a buffer solution (McDougall's artificial saliva) with no urea included, gassed with carbon dioxide, and continuously stirred on a hot plate. To each in vitro tube containing 0.5 g of ground prairie hay, 1 mL of Zn solution, 1 mL of Mn solution, 1 mL of a urea solution (30 mg urea N/L), and 40 mL of the ruminal fluid-buffer mixture were added. Concentrations of Zn in the added Zn solutions were 0, 215, 430, 645, and 860 ppm; diluted in 43 mL, supplemental concentrations of Zn provided the equivalent of 0, 5, 10, 15, or 20 ppm Zn in the incubation fluid. Similarly, the Mn solution contained 0 and 4,300 ppm Mn so that either 0 or 100 ppm Mn was added. Carbon dioxide was bubbled into each tube initially to establish an anaerobic environment. Separate duplicate sets of 100-mL polypropylene incubation tubes and 100-mL glass vials were used to measure urea degradation and IVDMD, respectively. Separate systems were used to avoid repeatedly opening and sampling of tubes being incubated for IVDMD measurement. The incubation tubes were outfitted with rubber stoppers through which glass tubes passed that were equipped with rubber policemen slit to permit gas release; glass vials were sealed with aluminum-rubber caps. Both were maintained at 39°C in a water bath. Duplicate tubes were incubated for a single-stage 48-h IVDMD (Tilley and Terry, 1963). For urea analysis, 1-mL aliquots were removed from each vial 0, 60, 120, and 180 min after incubation began and transferred into centrifuge tubes immersed in ice to stop fermentation and centrifuged at 13,600 $\times g$ for 1 min and frozen. Twenty-microliter subsamples of the supernatant fluid from each vial were analyzed colorimetrically for residual urea using a blood urea N kit from Sigma Chemical Co. (Crocker, 1967) that measured urea directly, not ammonia, with a Gilford Response UV-VIS spectrophotometer at a wavelength of 540 nm. Note that ureolysis was quantified directly as disappearance of urea, not accumulation of ammonia, as often has been used previously as an indirect index of ureolysis. This was an attempt to avoid attributing changes in concentration of ammonia strictly to altered ureolysis, when, in fact, ammonia concentration can be altered by additional factors such as increased or decreased utilization of ammonia by ruminal microbes.

Statistical Analysis. Data from the in vitro experiment were analyzed as a completely randomized design with a factorial arrangement of treatments using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). For IVDMD, the effects of treatment levels for Zn, Mn, and the Zn × Mn interaction were included in the model. Sampling date (two for IVDMD and three for ureolysis) nested within the interaction of Zn × Mn was used as the error term to test main effects. For the urea N concentration measurements, the design included a split-plot for the different incubation periods in time. The error term to test the time effect and the interactions of time with treatment (Zn or Mn) was sampling date nested within the three-way interaction of time × Zn × Mn. Preplanned orthogonal linear, quadratic, and cubic contrasts were used to interpret effects of Zn concentration on IVDMD of prairie hay and on urea degradation at specific times. The correlation between IVDMD at 48 h and urea hydrolysis by 48 h also was calculated.

Experiment 2

In an attempt to achieve mineral concentrations in the rumen similar to those employed in Exp. 1, we calculated the daily Zn dose needed to achieve maximum Zn concentrations similar to those used in vitro based on estimated feed DMI, ruminal volumes, and

turnover. However, as noted by Kennedy et al. (1993), Zn forms complexes with organic matter and particulate matter in the rumen. Nevertheless, we wanted to avoid excess ruminal Zn concentrations at any time. We calculated that the daily dosage of Zn needed to increase the Zn concentration in ruminal fluid by a maximum of 18 ppm (90% of the 20 ppm dosage noted by Martinez and Church [1970] as being detrimental) was 2.26 g Zn. This dosage is equivalent to a dietary dry matter concentration of 470 ppm Zn and can be contrasted with the maximum tolerable concentration suggested for beef cattle (NRC, 1996) of 500 ppm of Zn. We also employed two different urea levels, 45 and 90 g urea/d.

Animals and Treatments. Six ruminally cannulated heifers with a mean BW of 363 kg were allocated randomly to individual pens. These animals were supplied with 2% (DM basis) of their average BW daily of chopped prairie hay (5.1% CP on a DM basis). After hay consumption reached a plateau, daily feed DMI was limited to 4.8 kg DM, an amount 10 to 20% below the maximum DMI measured. Hay was provided in two equal meals daily at 0900 and 1700. The heifers were allotted randomly to squares that in turn were randomly allocated to one of the two levels of supplemental urea, 45 or 90 g/d. The same animals received each level of urea continuously during the experiment. This action was purposeful because, by confounding animal with square, statistical precision for detecting response to Zn and the Zn-urea interaction was increased. Zinc chloride was dosed intraruminally at rates of 0.14, 1.20, or 2.26 g; these amounts provided supplemental Zn concentrations estimated to be equivalent to approximately 30, 250, and 470 ppm. Identical Zn levels were used in both Latin squares. These Zn levels were designed either 1) to meet the minimum dietary Zn requirement (30 ppm in the diet) suggested by NRC (1996), 2) to provide a maximum ruminal concentration of Zn (18 ppm of ruminal contents) estimated to be 10% below the maximum ruminal concentration tolerated by bacteria suggested by Martinez and Church (1970), or 3) to provide a level intermediate (250 ppm in the diet) between these minimum and maximum dosages. To avoid any possibility of a deficiency of Mn, Mn chloride was added to all diets to increase the dietary Mn concentration by 40 ppm. The daily dosage of urea, Zn chloride, and Mn chloride for each heifer was placed directly into the rumen via the ruminal cannula once daily immediately before the morning feeding each day of the trial. Veterinarians at the College of Veterinary Medicine conducted ruminal fistulation of heifers, and the Institutional Animal Care and Use Committee approved all procedures.

Sampling Procedures and Determinations. Each period of the Latin square was divided into an initial adaptation period (d 1 through 7), sampling on d 8 (at 2, 4, 6, 12, 18, 21, and 24 h), and measurement of prairie hay consumption and digestibility (d 9 through 16). On d 9 through 16, each animal was given ad libitum access to prairie hay. Ruminal fluid, obtained through the ruminal cannula of each heifer, was filtered through four layers of cheesecloth, pH of the filtrate was measured immediately, and a 100-mL subsample of the filtrate was acidified with 2 mL of a 20% sulfuric acid solution and frozen. Later, this strained ruminal liquor was centrifuged for 15 min at 16,000 $\times g$ and the supernatant liquid was analyzed for minerals (Ca, P, Mg, Na, K, S, Zn, Mn, Cu, Fe, Cr, Co, Se, and Mo), NH_3 N, and VFA concentrations. Fecal grab samples were collected twice daily (0900 and 1700) on d 10 through 16.

Samples of hay, orts, and fecal material were collected, composited within animal and period, and dried at 55°C. After drying, each sample was ground through a 2-mm screen in a Wiley mill (Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA) and stored for later analysis. The DMI was computed by subtracting dry orts weight from dry weight of prairie hay provided. Dry matter digestibility (**DMD**) was calculated by using lignin as an inherent marker, and digestible DMI (**DDMI**) was calculated as the multiple of DMI and DMD. Though imperfect as a digestibility marker, relative estimates of digestibility should be accurate unless supplemental Zn altered lignin disappearance.

Laboratory Analyses. Concentrations of minerals in the supernatant fluid from ruminal contents were determined using an Inductively Coupled Plasma Spectrometer (Spectroflame FTM-08, Spectro Analytical Instruments, Fitchburg, MA) relative to stock solution standards (High Purity Standards Co., Charleston, SC). A minimum of four points was used for regression calibration of the instrument.

For measurement of ruminal NH_3 N concentration, 50-μL aliquots were analyzed colorimetrically at 630 nm using a Beckman spectrophotometer (Beckman, DU 64, Beckman Instruments, Fullerton, CA) following the procedure of Broderick and Kang (1980). Ruminal fluid VFA concentrations were determined using a Perkin-Elmer Autosystem gas chromatograph (Perkin-Elmer 9000 Model Series, Norwalk, CN) equipped with a Megabore DB-FFAP liquid phase column, using helium as a carrier and 2-ethylbutyric acid as an internal standard. Samples of prairie hay, orts, and feces were analyzed for DM and ADL (Goering and Van Soest, 1970).

Statistical Analysis. Analyses of variance were performed using the GLM procedure of SAS. In this experiment, urea was confounded with square (and thereby with animal). The validity of imposing different treatments in different Latin squares and testing for main plot effects (square effect) by animal within square and the validity of testing subplot treatment effects and the interaction between the subplot and the main plot factor by the residual error term is valid statistically. However, power for testing the main plot effect, in this case urea level, was very limited because it was tested only by the animal-within-square component. As a result of this limited statistical power and high likelihood of failing to detect a true difference, statistical effects of the dose level of urea, though legitimately analyzed

statistically, were not tested independently but combined with animal effects in the statistical model employed. After removal of animal (now representing both the urea or square as well as the animal within urea) effects (5 df), the remaining subplot effects (period with 2 df, Zn concentration with 2 df, and the Zn × urea interaction with 2 df) were tested by the residual subplot error. For measurements repeated across time, including ruminal pH and concentrations of minerals, NH_3 N and VFA, data were treated as repeated measures over time and analyzed using the repeated measures option of GLM. When the time × Zn or time × Zn × urea interaction was detected as being significant ($P < 0.10$), time periods were analyzed individually to determine the specific time period(s) when differences occurred. Linear and quadratic orthogonal responses to Zn concentration were examined through contrast statements for unequally spaced Zn levels. In addition, preplanned orthogonal contrasts were used to compare treatment means for the early (2 to 6 h) vs the late (12 to 24 h) postprandial periods after urea dosing.

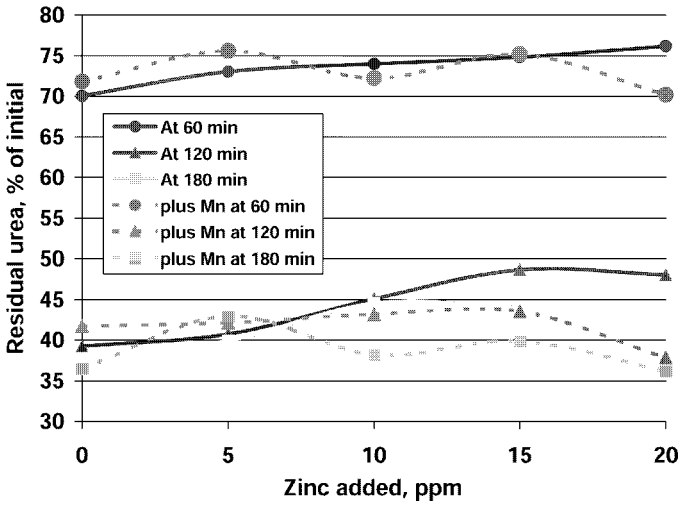

Figure 1. Concentration of urea in tubes after 60, 120, and 180 min of in vitro incubation with various amounts of Zn added with or without added Mn in Exp. 1.

Results and Discussion

Experiment 1

IVDMD in Prairie Hay. For IVDMD, the Zn × Mn interaction was not significant ($P = 0.93$). However, as Zn concentration was increased, IVDMD decreased linearly ($P < 0.03$); 44.7, 44.3, 43.8, 43.2, and 40.9% of DM had disappeared at 24 h with 0, 5, 10, 15, and 20 ppm added Zn. Addition of Mn increased ($P < 0.02$) IVDMD (44.7 vs 42.0 with 100 vs 0 ppm added Mn). Chamberlain and Burroughs (1962) reported that cellulose digestibility was reduced when Mn was omitted from an in vitro incubation medium. Using washed suspensions of ruminal microorganisms, Martinez and Church (1970) found that additions of 20 ppm of Zn or of 100 ppm of Mn decreased cellulose digestibility by 31 and 24%, respectively. These results support the suggestion that an elevated concentration of Zn can depress fiber digestibility. However, the increases in IVDMD noted with addition of Mn in our study have not been reported previously.

Ureolysis. As expected, mean urea N concentration decreased linearly ($P < 0.01$) over time, averaging 38.0, 27.6, 16.8, and 15.2 mg/dL (SE = .45) at 0, 60, 120, and 180 min, respectively; the rate of urea degradation was slowest from 120 to 180 min. No reason for less ureolysis at the later time interval is apparent, although product inhibition may have occurred. Because the interaction between time and Zn concentration approached significance ($P < 0.09$), urea disappearance at specific time intervals was analyzed individually. Results are plotted in Figure 1. Concentration of residual urea, expressed as a percentage of urea present in tubes initially, was not altered by either Zn or Mn at 60 min ($P > 0.37$). However, at 120 min, a linear trend ($P < 0.06$) for increased residual urea was apparent with added Zn. A Zn × Mn interaction ($P < 0.04$) also was detected in which added Mn counteracted the effect of added Zn. By 180 min, the effect of added Zn on concentration of residual urea was quadratic ($P < 0.02$), although again an independent interaction between Zn and Mn ($P < 0.04$) remained in which added Mn counteracted the effect of added Zn. The amount of additional Zn that, in the absence of added Mn, resulted in the greatest retention of urea present initially, based on solving appropriate quadratic regression equations, was 24.4 ppm at 60 min, 20.6 ppm at 120 min, and 13.9 ppm at 180 min of incubation.

Various minerals, including Cu, Zn, Cd, Sr, Ca, Co, Mn, Ba, and Mg, have been reported to inhibit ruminal ammonia accumulation from urea in vitro (Spears and Hatfield, 1978), but whether these responses are due to decreased ureolysis or increased ammonia utilization by ruminal microbes is not certain. Similarly, addition of Ca, Mg, Fe, Mn, and Zn individually or in various combinations has been reported to reduce in vitro NH_3 accumulation at 3 h in the presence of urea by up to 20% (Rodriguez et al., 1993a). Added Zn or Zn plus Mn inhibited NH_3 accumulation in the presence of urea in vitro by 24 and 18%, respectively (Rodriguez et al., 1993b). These results support the concept that either ureolysis or ammonia utilization can be modified by addition of minerals to ruminal fluid. Results from our experiment indicate that ureolysis is inhibited by Zn, though an unexplained interaction between Zn and Mn was apparent. In this study, ureolysis at 180 min was not correlated ($r = 0.02$; $P = 0.95$) with IVDMD at 48 h.

Experiment 2

Prairie Hay Utilization. When given free-choice access to feed, heifers had average DMI of 4.94 kg daily, or 1.36% of mean BW. This level of hay consumption was

Table 1. Daily prairie hay DMI and digestibility by heifers receiving different levels of supplemental dietary Zn (Exp. 2)

	Diet zinc concentration, ppm				Zinc response, $P <$	
Item	30	250	470	SE	Linear	Quadratic
DMI, kg	5.26	4.63	4.92	0.27	0.42	0.22
DMI, g/BW$^{.75}$	61.4	54.6	58.1	2.06	0.49	0.23
DM digestibility, %	48.5	49.1	42.8	2.53	0.16	0.31
Digestible DMI, kg/d	2.56	2.26	2.02	0.17	0.07	0.89
Digestible DMI, g/BW$^{.75}$	29.9	26.7	24.3	3.17	0.08	0.86

lower than the 2% offered and was only slightly above the 4.75 kg of DM fed during the restricted DMI phase of each period. Level of supplemental Zn did not significantly alter DMI, DMD, or DDMI (Table 1). However, daily intake of DDMI, either expressed as kilograms or as grams per kilogram BW$^{.75}$, tended ($P < 0.07$) to be linearly reduced by added Zn. This was largely due to a tendency for reduced diet digestibility at the highest Zn intake. Elevating Zn in vitro to 20 or 30 ppm has decreased in vitro cellulose digestion (Martinez and Church, 1970). Puls (1990) indicated further that excessive dietary Zn reduces feed consumption by sheep.

Ruminal Fluid Measurements. The only mineral whose concentration in centrifuged ruminal fluid was affected by Zn addition was Zn; it increased linearly ($P < 0.01$) with Zn supplementation to means of 2.26, 7.6, and 11.6 ppm Zn with 30, 250, and 470 ppm supplemental dietary Zn, respectively. Postprandial time effects on ruminal concentrations of certain minerals were detected with linear increases ($P < 0.02$) with postprandial time (from 2 to 24 h only) and higher ($P < 0.01$) concentrations during the first four than during the final three sampling times noted for P, K, Fe, S, and Na. Presumably, these changes reflect the balance between minerals being solubilized from the forage in the rumen, input of minerals from saliva, and removal via absorption and passage to the omasum. Concentration of Zn tended ($P < 0.06$) to decrease with postprandial time (from time 2 to time 24 h only; Figure 2) and was lower ($P < 0.04$) during the final three than during the earlier four sampling times.

With steers fed diets containing 50% roughage at 2-h intervals, Froetschel et al. (1990) reported that addition of 1,142 ppm Zn to the diet increased Zn concentration in ruminal fluid from approximately 0.5 to 4.8 ppm. Similarly, Kennedy et al. (1993) noted that increasing the dietary Zn concentration from 27 to approximately 115 ppm by adding Zn oxide or a polysaccharide complex of Zn with the Zn supplement fed once daily caused Zn concentrations to peak following supplement intake; Zn concentrations in ruminal fluid were 1.9, 5.0, and 7.2 ppm at 2 h after supplement was consumed. The solubility of minerals from forages into ruminal contents depends on plant maturity (Kabaija and Smith, 1988), the mineral itself, the fraction of the plant to which the mineral is associated, ruminal pH, and type of forage (Emanuele and Staples, 1990, 1994).

Interactions between sampling time and dietary Zn supplementation level approached significance both for ruminal pH ($P < 0.08$) and ruminal ammonia concentration ($P < 0.07$). However, no interaction between urea and Zn supplementation was detected for either pH ($P = 0.97$) or ammonia concentration ($P = 0.43$). Consequently, linear and quadratic responses to Zn concentration were analyzed within each sampling period across both Latin squares. Results for pH are reported in Table 2 and Figure 3, and ammonia N concentrations are reported in Table 3 and Figure 4. At 2 h after dosing, pH was linearly decreased ($P < 0.05$) by added Zn, whereas at 6 h after dosing pH was linearly increased ($P < 0.05$) by added Zn. At no other time did Zn supplementation significantly ($P = 0.19$ to 0.96) alter ruminal pH. At 2 h after dosing, ruminal ammonia was linearly decreased ($P < 0.01$) by added Zn, but no significant effects were detected at other sampling times. Because ammonia, with a pK of 9.3, will act as a base at neutral pH, the higher ammonia concentration at 2 h can explain the increase in ruminal pH at 2 h. The higher ammonia concentration and the lower ruminal pH at 2 h after feeding with higher doses of Zn can be interpre-

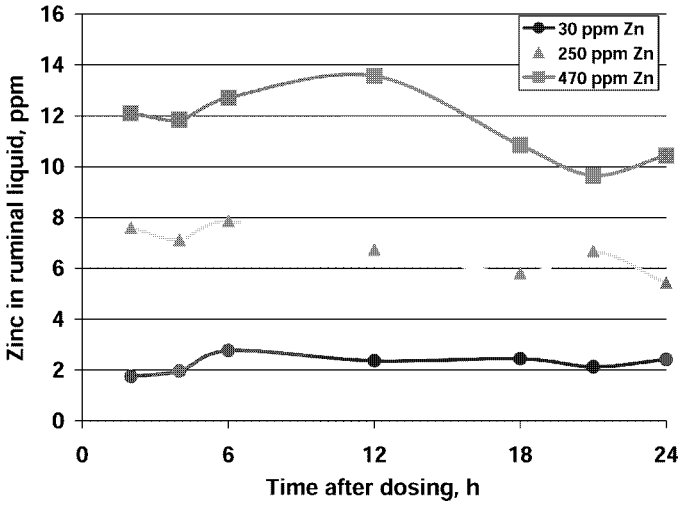

Figure 2. Ruminal concentration of Zn at different time intervals after addition of supplemental dietary zinc (30, 250, and 470 ppm) in Exp. 2. Values for prefeeding samples (24 h) are repeated at the initial prefeeding time (0 h) to illustrate changes over the full day.

Table 2. Ruminal pH at various times after heifers fed prairie hay received ruminally dosed urea and Zn (Exp. 2)

	Zinc concentration, ppm				Zn response, $P <$	
Time after dosing	30	250	470	SE	Linear	Quadratic
2 h	7.18	6.95	6.86	0.096	0.05	0.55
4 h	7.03	7.19	7.03	0.075	0.96	0.14
6 h	6.77	6.87	6.94	0.053	0.05	0.89
12 h	6.52	6.58	6.66	0.070	0.19	0.90
18 h	6.71	6.70	6.69	0.090	0.92	0.98
21 h	6.82	6.72	6.77	0.064	0.61	0.43
24 h	6.68	6.62	6.71	0.084	0.81	0.55

ted to suggest that added Zn inhibited urea hydrolysis to ammonia. The delayed peak for ruminal pH to 6 h noted with the highest amount of supplemental Zn suggests that ureolysis was postponed but not completely inhibited by this level of supplemental Zn.

These results confirm a previous suggestion that Zn at concentrations of 130 to 1,300 ppm decreases NH_3 N accumulation from urea during in vitro incubation of ruminal fluid from sheep (Spears and Hatfield, 1978) and that Zn supplementation decreases ruminal NH_3 concentrations in sheep fed urea-supplemented, low-quality roughage (Rodriguez et al., 1995). Through decreasing rate of NH_3 release from urea, supplemental Zn may decrease the incidence of urea (ammonia) intoxication and thereby may reduce ammonia loss associated with absorption from the rumen. This, in turn, could help maintain microbial activity and extent of microbial protein synthesis in the rumen (Johnson, 1976; Mizwicki et al., 1980).

Urease is produced by a wide variety of ruminal microbes, and urease activity can be altered by various dietary factors (Wozny et al 1977) as well as by concentrations of ammonia, urea, and certain minerals (e.g., Spears and Hatfield, 1978; Wallace and Cotta, 1988). Whether elevated Zn concentrations depress urease activity directly or might inhibit growth and reduce the population of ureolytic bacteria in the rumen needs further investigation.

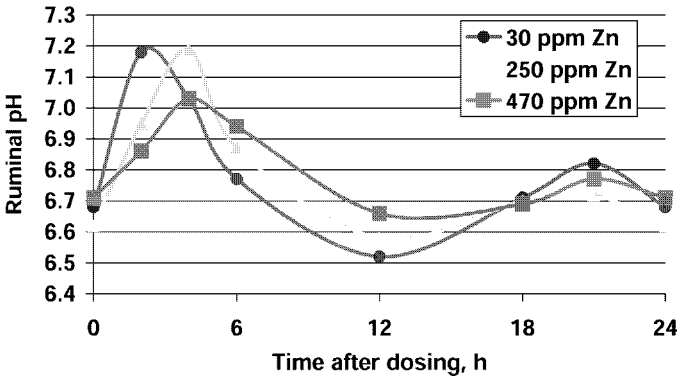

Figure 3. Ruminal pH at various postprandial times with 30, 250, or 470 ppm zinc added to the diet in Exp. 2. Values for prefeeding samples (24 h) are repeated at the initial prefeeding time (0 h) to illustrate changes over the full day.

Mean molar proportions of ruminal VFA were 60.2% acetate, 15.1% propionate, 0.4% isobutyrate, 6.2% butyrate, 0.5% isovalerate, and 1.0% valerate, whereas total VFA concentration averaged 83.4 m*M*. The molar proportion of propionate was increased linearly ($P < 0.02$) and quadratically ($P < 0.01$) by added Zn, with 13.0, 15.5, and 16.9% of VFA being propionate with 30, 250, and 470 ppm supplemental dietary Zn. No other effects of supplemental Zn were detected. These increases in the molar proportion of propionate resulted in linear ($P < 0.02$) and quadratic ($P < 0.05$) decreases in the acetate:propionate ratio, with ratios of 4.71, 3.68, and 3.83 with 30, 250, and 470 ppm supplemental dietary Zn, respectively. No interactions between Zn supplementation and sampling time were detected, but sampling time affected molar proportions of acetate, isobutyrate, valerate, and the acetate:propionate ratio of ruminal fluid ($P = 0.02$). The molar proportion of isobutyrate increased over time but the molar proportions of acetate, valerate, and the acetate:propionate ratio all decreased. Total VFA content also tended to decline ($P < 0.09$) with postprandial time. Various dietary compounds, including the ionophore antibiotics (monensin, lasalocid, and salinomycin), change the molar proportions of ruminal VFA through inhibition of certain Gram-negative, hydrogen-producing ruminal microbes (Van Soest, 1982). Thereby, they increase the relative proportion of propionate (Van Nevel and Demeyer, 1988). Perhaps through altering K or Na transport, elevated Zn concentrations may alter microbial species in a fashion similar to ionophores. Another inhibitor of urease activity, N-(n-butyl) thiophosphoric triamide, was reported to decrease the acetate:propionate ratio after 6 h of in vitro incubation with ruminal fluid (Ludden et al., 1998). Froetschel et al. (1990) noted that Zn supplementation (1,142 ppm) of a 50% concentrate diet, when fed every 2 h, increased the molar proportion of propionate and decreased the acetate:propionate ratio of ruminal samples. Like ionophores, supplemental Zn may alter the populations of specific ruminal microbes and thereby change rates of production of different VFA. Karr et al. (1991) indicated that Zn at

Table 3. Ruminal ammonia nitrogen concentration (mg/dL) at various times after heifers fed prairie hay received ruminally dosed urea and Zn (Exp. 2)

	Zinc concentration, ppm				Zn response, $P <$	
Time after dosing	30	250	470	SE	Linear	Quadratic
2 h	56.0	43.3	34.8	3.69	0.01	0.67
4 h	34.4	42.2	33.9	8.52	0.96	0.47
6 h	15.5	22.3	20.9	4.39	0.42	0.47
12 h	2.4	2.9	5.4	2.29	0.38	0.73
18 h	1.4	1.3	1.1	0.44	0.71	0.95
21 h	2.2	1.8	1.7	0.30	0.28	0.60
24 h	1.2	1.2	0.82	0.32	0.43	0.60

concentrations of 10 to 80 ppm inhibited growth of selected strains of ruminal bacteria; strains producing thiol proteases seemed to be more sensitive to Zn. Whether effects of Zn on ruminal VFA patterns are additive with ionophores remains unknown.

An increased proportion of propionate in ruminal VFA that leads to an increased energetic efficiency of ruminal fermentation might explain the consistent benefits obtained when chelated Zn supplements and Zn-treated protein sources are fed. Treatment of soybean meal with Zn salts at 10,000 to 20,000 ppm will decrease degradation of protein as measured in situ (Cecava et al., 1993) or by incubation with ficin (Poos-Floyd et al., 1985). This presumably is due to insolubilization of the soy protein but also acts perhaps through alteration in the activity of proteolytic microbes. Zinc treatment of soybean meal also enhanced efficiency of N utilization by growing calves (Britton and Klopfenstein, 1986). Diets containing Zn-treated soybean meal often contain over 150 ppm Zn, and, if released in the rumen, these may elevate ruminal Zn concentrations. Cecava et al. (1993) detected no increase in ruminal escape of dietary protein with Zn-treated soybean meal when compared with a basal soybean meal diet that was supplemented with a similar amount of Zn. Alterations in VFA patterns also might explain why feedlot consultants often formulate feedlot diets with Zn present at 300 ppm or more (Galyean, 1996), levels considerably higher than the 30 ppm supposedly required nutritionally (NRC, 1996) by finishing beef cattle. In our experiments, Zn was added as the chloride salt; bioavailability may be higher or lower for other sources of Zn (Baker and Ammerman, 1995), although effects of source of Zn on ruminal concentrations, that need not be proportional to bioavailability, may be more closely related to alterations in fermentation kinetics.

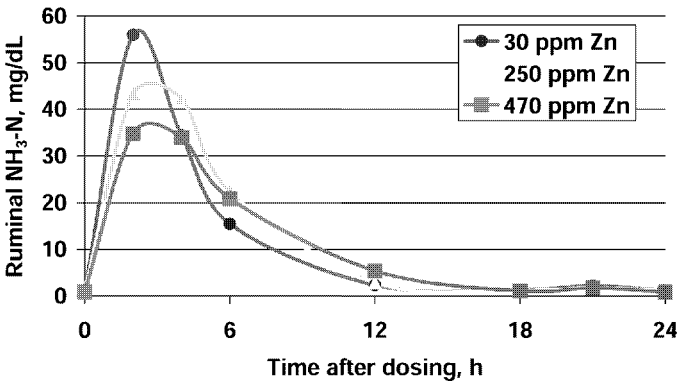

Figure 4. Ruminal ammonia N at various postprandial times with 30, 250, or 470 ppm zinc added to the diet in Exp. 2. Values for prefeeding samples (24 h) are repeated at the initial prefeeding time (0 h) to illustrate changes over the full day.

Overall, decreased urea degradation in vitro and temporal alterations in ruminal pH and ammonia concentrations associated with added Zn support previous reports that elevated Zn concentration inhibits urease activity of ruminal fluid. Whether these are direct effects on enzymatic activity or they inhibit ruminal microbes that produce urease is not certain, although the increase in the proportion of ruminal propionate and the decrease in the acetate:propionate ratio suggest that ruminal fermentation patterns are altered by supplemental Zn. Conversely, the antagonism between Mn and Zn on urease activity closely parallels the antagonism between these two ions with at least five other isolated enzymes (Dixon and Webb, 1964), so perhaps Zn directly reduces urease activity. Decreased IVDMD with high Zn concentrations supports the concept that Zn can inhibit activity of fiber-digesting ruminal microbes. Widespread accessibility of Zn salts and its low cost for the dose needed (equivalent to an intake of 1.3 to 2.6 g/d) makes Zn supplementation potentially beneficial both to inhibit NH_3 release and to alter ruminal fermentation patterns. However, supplying more than 250 ppm Zn may decrease digestibility, particularly of fiber, as indicated by the in vitro study and supported by the trend noted in the in vivo trial. High intakes of Zn can alter absorption or retention of other minerals (Puls, 1990; NRC, 1996), so care in balancing other minerals, particularly Cu, should be exercised.

Implications

Supplementation with Zn at low concentrations (10 to 15 ppm in the incubation fluid) inhibited in vitro urea hydrolysis and retarded ammonia accumulation

in the rumen. When daily doses of supplemental Zn equaled or exceeded the dietary equivalent of 250 ppm, the molar proportion of ruminal propionate was increased and the acetate:propionate ratio was decreased. Supplementation of the diet with Zn at a dietary level of 250 ppm, to increase the Zn concentration in ruminal liquid to approximately 7 ppm, may prove beneficial to decrease the potential for urea toxicity and to favorably alter the pattern of volatile fatty acids in the rumen. However, adding 470 ppm Zn, to achieve a concentration in ruminal liquid of 12 ppm, tended to depress digestibility.

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