EFFECTS OF SUPPLEMENTAL ZINC AND MANGANESE ON *IN VITRO* UREA DEGRADATION AND PRAIRIE HAY DISAPPEARANCE

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Story in Brief

Rates of disappearance of urea and prairie hay *in vitro* with various zinc (Zn) and manganese (Mn) concentrations were appraised. Five concentrations of Zn (0, 215, 430, 645 and 860 ppm) and two of Mn (0 and 4300 ppm) were tested in a triplicated 2 x 5 factorial experiment. Each fermentation tube contained .5 g of prairie hay, 1 mL of Zn and 1 mL of Mn solution (both as the chloride), 1 mL of an urea solution (35g/L), 20 mL of ruminal fluid, and 20 mL of McDougall□s artificial saliva. These mineral concentrations equate to daily steer intakes of 0 to 2.62 g Zn, 0 to 13.10 g Mn, and 100 g of urea. Ruminal fluid was obtained from three 1,189 lb cannulated steers 24 h after the last meal of 15 lb of prairie hay plus 1 lb of a supplement (89.5% ground corn, 10 % urea, .5 % NaCl). Tubes were incubated at 39°C to measure dry matter disappearance (IVDMD) in a single-stage 48 h period. One mL samples were removed from each tube at 0, 60, 120 and 180 min after incubation began. Subsamples were analyzed colorimetrically for residual urea. Incubations were repeated on three different dates. IVDMD of prairie hay was increased by added Mn but decreased linearly with added Zn. Urea concentration decreased over time (38.0, 27.6, 16.8 and 15.2 mg/dL at 0, 60, 120 and 180 min). Analyzed within specified time intervals, added Zn linearly decreased ureolysis at 60, 120, and 180 min although an interaction with Mn was apparent at 120 and 180 min in which ureolysis was less inhibited by Zn when Mn was added. Results support previous observations that added Zn retards conversion of urea to ammonia.

(Key Words: Zinc, Manganese, Dry matter disappearance, Ureolysis.)

Introduction

Low quality roughage is used to maintain or to support intermediate rates of production by ruminants. Because low quality forages often are low in N, they must be supplemented for optimum intakes and performance by ruminants (Hunt et al., 1989). Urea, an alternative N source for ruminants, usually is a cheaper source of N than other supplements. Increasing the amount of ruminally degraded protein (DIP) by adding urea sometimes increases forage intake and digestibility. In one recent study, urea could replace up to 40% of the supplemental DIP without affecting forage intake and digestion by beef cattle consuming low quality forages (Vazant, 1996).

Problems related to urea use in ruminants include very rapid hydrolysis to ammonia within the rumen that can decreases N retention and result in toxicity, low feed intakes, and reduced productivity. Ruminal ammonia and blood urea concentrations generally increase with N intake. A rapid rise in blood urea-N indicates that ruminal N is being inefficiently utilized for ruminal protein synthesis (Arelovich et al., 1992). This buildup of urea in blood indicates that ammonia is absorbed from the rumen at a rate higher than it is being used, a condition that can cause toxicity. Urea could be used more safely and might prove useful in larger amounts if its ammonia release

rate in the rumen were prolonged. Minerals such as Cu, Zn, Cd, Sr, Ca, Co, Mn, Ba and Mg inhibit ruminal urease activity (Spears and Hatfield, 1978). If supplied with urea, these minerals might reduce ruminal urea degradation rate and improve its utilization

In vitro ruminal urease activity was reduced by Zn or Mn plus Zn to about 20% less of the control treatments in sheep (Rodriguez et al., 1993). Results from an *in vivo* trial showed that N balance and weight gain was improved when urea supplements contained Zn or Mn plus Zn (Rodriguez et al., 1995). In this experiment, the rates of disappearance of urea and prairie hay were determined *in vitro* with various Zn and Mn concentrations.

Materials and Methods

Rates of disappearance of urea and of prairie hay *in vitro* at various Zn and Mn concentrations were appraised. Five concentrations of supplemental Zn (0, 215, 430, 645 and 860 ppm) and two of Mn (0 and 4300 ppm) were tested in a 2 x 5 factorial experiment. Preliminary *in vitro* determinations indicated that response to Zn was greater than to Mn, so only two Mn concentrations were tested. Ruminal fluid was obtained from three 1,189 lb. rumen cannulated steers fed a daily diet of 15 lb. of prairie hay plus 1 lb. of a supplement (89.5% ground corn, 10 % urea, .5 % NaCl). Amounts of Zn and Mn to use were estimated from predictions of rumen capacity and turnover rate, mineral intake and availability from the hay and the mineral supplements, and mineral tolerance values. Based on these calculations, *in vitro* mineral concentrations we used should equal ruminal supplies equal to supplemental daily intakes of 0 to 2.62 g Zn and 0 to 13.10 g Mn, and 100 g of urea (Table 1). The maximum mineral concentrations tested is equal to those reported to depress activity of ruminal bacteria (Martinez and Church, 1970)

About 750-mL of ruminal fluid, obtained from each steer 24 h after the last meal, was mixed and used as a source of inoculum. Each *in vitro* tube contained .5 g of prairie hay, 1 mL of Zn and 1 mL of Mn solution (both as chlorides), 1 mL of an urea solution (35g/L), 20 mL of ruminal fluid and 20 mL of McDougall□s artificial saliva. For zero supplemental Zn or Mn, 1-mL of distilled water replaced the mineral solutions. Duplicate tubes were incubated at 39° C for a single-stage 48 h *in vitro* dry matter disappearance. One mL subsamples were removed from each tube at 0, 60, 120 and 180 min after incubation began, stored into a 1-mL centrifuge tubes immediately immersed on ice, centrifuged at 12,400 g for one minute and kept refrigerated for urea analysis. Separate tubes were used for *in vitro* dry matter disappearance (IVDMD) and urea determinations. Samples were analyzed colorimetrically for residual urea and gravimetrically for residual dry matter. Incubations were repeated on three different dates.

The experiment was analyzed statistically as a completely randomized factorial arrangement of treatments for IVDMD with a split plot design in time. Data on urea-N concentration were analyzed as repeated measures for various sampling time, using urea-N concentration at time 0 as a covariate. Pre-planned orthogonal linear, quadratic and cubic contrasts were used to interpret mineral effects on IVDMD of prairie hay and urea degradation at specific times. The statistical analysis was performed by following the procedure of SAS (1985).

Results and Discussion

In vitro dry matter disappearance of prairie hay is presented in Figure 1. Overall, Mn addition increased ($P < .05$) dry matter disappearance. This may be a consequence of the marked linear decrease ($P < .05$) in IVDMD with the added Zn especially at the highest Zn level. The interaction between Zn and Mn on IVDMD was not significant.

Mean urea-N decreased over time, being 38.0, 27.6, 16.8 and 15.2 mg/dL (SE= .45) at 0, 60, 120 and 180 min, respectively ($P = .0001$); rate of urea degradation was slowest from 120 to 180 min. Residual urea-N values for the various are reported in Table 2. When analyzed within specified time intervals, added Zn linearly increased residual urea at 60, 120, and 180 min ($P < .08$; .10 and .02, respectively) although interactions with Mn were apparent ($P < .03$) at 120 and 180 min. To illustrate the general trends of all treatments, data on urea-N concentration were transformed into percentages of the initial urea concentration. Percentages of *in vitro* urea lost after 120 minutes incubation period are shown in Figure 2.

Urea disappeared rapidly for the first 120 minutes and less rapidly thereafter. Ureolysis appeared to be inhibited less by Zn when Mn was supplemented; thus, higher urea-N concentrations were found in those samples containing the highest Zn levels, except when combined Mn also was added. Nevertheless, absolute values varied with sampling dates. Although procedures were standardized with respect to differences in rumen fluid and temperature, small differences in sampling time or agitation of tubes could contribute to this variability.

Rate of IVDMD was not related to rate of ureolysis. Dry matter disappearance decreased linearly as Zn concentration was increased and tended to increase with Mn. A decreased rate of urea degradation supports previous measurements of the effect of Zn concentration on *in vitro* ammonia accumulation.

Implications

Urea hydrolysis by ruminal fluid was inhibited by moderate levels of supplemental zinc. This potentially could increase usefulness of urea by ruminants. Further studies are needed to determine whether similar effects occur in the rumen where ruminal turnover, recycling of urea, and microbial adaptation may be altered by supplemental minerals.

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Table 1. Mineral concentrations for the *in vitro* study and equivalent animal daily intake.

Mineral levels		Zn Cl_2[a] g/L	Mn Cl_2[b] g/L	Concentration[c] equivalent ppm	Daily intake[d] equivalent g/d
Zn ppm	Mn ppm				
0	0	.00	.00	.0	.00
215	0	.45	.00	5.0	.66
430	0	.90	.00	10.0	1.31
645	0	1.35	.00	15.0	1.96
860	0	1.80	.00	20.0	2.62
0	4300	.00	15.48	100.0	13.10

[a] Anhydrous zinc chloride.

[b] Manganese chloride crystallized with 4 molecules of water.

[c] Equivalent to the rumen concentration in the animal.

[d] Equivalent to total daily intake from the mineral supplement. Intake from hay is assumed to be negligible.

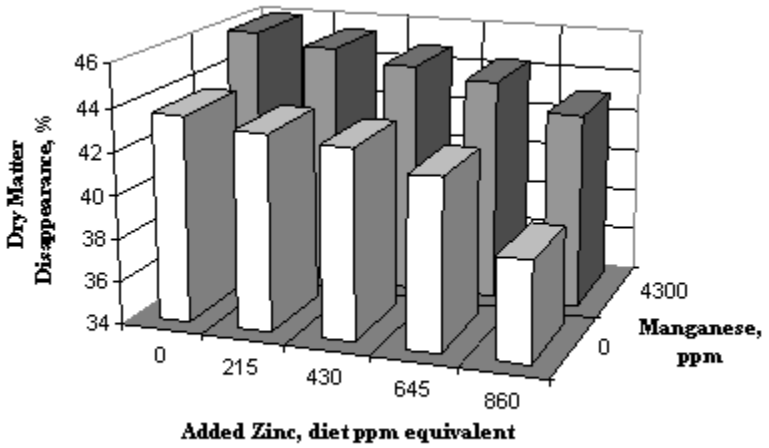

Figure 1. *In vitro* dry matter disappearance of prairie hay after 48-h incubation with different added Zn- Mn levels. Overall effect of Mn and linear of Zn (P <.05, SE = 1.002)

Table 2. *In vitro* urea-N concentration (mg/dL) with different levels of Zn and Mn at 0, 60, 120 and 180 minutes after *in vitro* incubation.

Time (min)[a]		0	60	120	180
Level[b], ppm		Urea-N mg/dL			
Zn	Mn				
0	0	39.09	27.22	15.58	14.77
215	0	40.54	29.53	16.97	15.90
430	0	38.50	28.00	21.17	16.92
645	0	37.41	27.90	18.39	16.42
860	0	36.58	27.84	17.75	15.65
0	4300	38.45	27.20	16.08	13.82
215	4300	37.47	28.09	15.78	16.01

430	4300	38.55	27.41	16.67	14.46
645	4300	35.55	26.66	15.73	14.21
860	4300	37.45	25.84	14.21	13.41

[a] Sampling time after incubation (P<.01).

[b] Added levels of Zn and Mn.

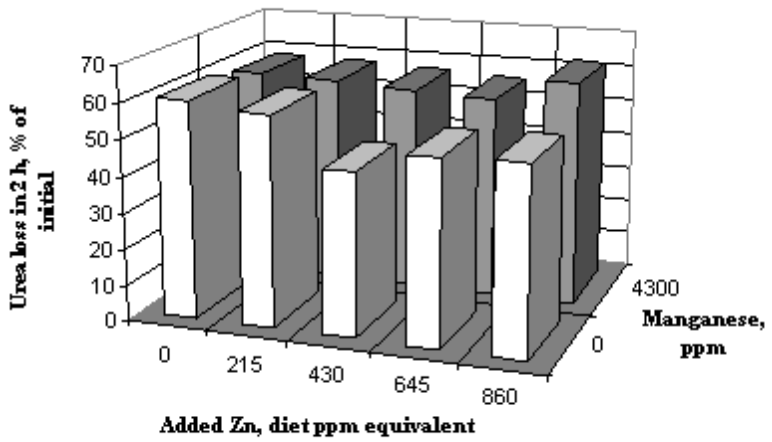

Figure 2. Percentage *in vitro* urea loss at 2 h with various amounts of added Zn and Mn.

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