Effects of Zinc and Sodium Monensin on Ruminal Degradation of Lysine-HCl and Liquid 2-Hydroxy-4-Methylthiobutanoic Acid*†

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ABSTRACT

Four nonlactating, mature, Holstein cows were fitted with ruminal cannula and used in a 4 × 4 Latin square-designed experiment to evaluate the impact of supplemental Zn and monensin on ruminal degradation of Lys and liquid 2-hydroxy-4-methylthiobutanoic acid (HMB). Cows were fed 4.54 kg (as fed) of alfalfa hay top-dressed with 4.54 kg (as fed) concentrate once daily. Concentrates were formulated to provide 0 or 500 mg/kg of Zn as ZnSO₄ and 0 or 40 mg/kg of monensin in the total diet. Zinc supplementation provided approximately 22-fold greater dietary Zn than estimated by NRC requirements. On d 14 of each period, cows were dosed via the rumen cannula with 50 g of HMB and 100 g of Lys-HCl, and the concentrations of Lys and HMB were monitored every 0.5 h for 8 h. Supplemental Zn tended to decrease the proportion of acetate in ruminal fluid postfeeding and increased the proportion of propionate in ruminal fluid postfeeding. Supplemental Zn increased mean fluid passage rate from the rumen. Monensin decreased the proportion of acetate and increased the mean proportion of propionate in ruminal fluid, resulting in a decrease in the ratio of acetate to propionate. Monensin also increased the mean fluid passage rate from the rumen. Neither Zn nor monensin affected the apparent rate of ruminal disappearance of

HMB or Lys. However, Zn and monensin interacted to alter the ruminal degradability of free Lys but not HMB. These data indicate that Zn and monensin may interact to alter ruminal degradability of free amino acids.

(**Key words:** monensin, zinc, amino acid degradability)

Abbreviation key: HMB = liquid 2-hydroxy-4-methylthiobutanoic acid, K_d = rate of disappearance, K_p = fluid turnover rate of the rumen, SBM = soybean meal.

INTRODUCTION

Methionine and Lys have been identified as the first limiting AA for milk and milk protein production (Schwab, 1996). Because of this, various strategies have been developed to protect these AA to prevent their ruminal degradation but still allow them to be absorbed at the small intestine (Chalupa, 1975). Because of the high rate of passage for soluble nutrients from the rumen of a high-producing dairy cow, significant amounts of AA fed in the free crystalline form may reach the intestine (Volden et al., 1998). Thus it may be possible to add dietary supplements that inhibit ruminal degradation of free AA and, therefore, increase the flow of these AA to the small intestine.

Supplemental Zn decreased the ruminal degradation of feed proteins (Froetschel et al., 1990; Arelovich et al., 2000). Supplemental Zn inhibited urease activity *in vitro* (Spears and Hatfield, 1978). Zinc may interfere with ruminal proteolysis in a similar manner. Additionally, Zn, along with other metal ions, can be used to precipitate proteins from an aqueous solution.

Ionophores, such as sodium monensin, also alter the ruminal degradation of feed proteins (Poos et al., 1979) and peptides (Bergen and Bates, 1984; Russell and

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Table 1. Ingredient composition of concentrate mixtures fed to cows.

Ingredient	– Zn		+ Zn	
	– Monensin	+ Monensin	– Monensin	+ Monensin
	% of DM			
Ground corn	61.46	61.46	61.46	61.46
Soybean meal	34.40	34.40	34.40	34.40
Molasses	3.00	3.00	3.00	3.00
Rock phosphate	0.40	0.40	0.40	0.40
Salt	0.38	0.38	0.38	0.38
Trace mineral supplement ¹	0.04	0.04	0.04	0.04
Vitamin supplement ²	0.03	0.03	0.03	0.03
Limestone	0.29	0.29	0.29	0.29
ZnSO ₄	0	0	0.27	0.27
Rumensin 80 ³	0	0.02	0	0.02

¹Contains 8.44% Ca, 6.65% P, 4.33% Mg, 2.64% S, 3,373 mg/kg of I, 125,873 mg/kg of Mn, 85,324 mg/kg of Zn, 16,195 mg/kg of Fe, 642 mg/kg of Co, and 24,369 mg/kg of Cu.

²Contains (per kg) 11,137 kIU of vitamin A, 2,784 kIU of vitamin D, 30,928 IU of vitamin E, and 680 mg of Se.

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Strobel, 1989; Chen and Russell, 1991). Monensin inhibits some proteolytic bacteria, protozoa, and fungi in the rumen (Russell and Strobel, 1989). Also, monensin may have a direct effect on protease and deaminase enzymes (Bergen and Bates, 1984). Additionally, monensin increased the apparent digestibility of Zn but decreased the concentration of Zn in ruminal fluid (Kirk et al., 1985). However, feeds containing monensin must be segregated from those fed to lactating dairy cows, lactating dairy goats, and horses, since the product currently is not approved for use in those species.

We are unaware of any previous research that has investigated the effects of high dietary Zn concentrations (approximately 22-fold greater than requirements) and monensin on ruminal degradation of free AA (not feed bound). Therefore, this experiment was designed to investigate the effects of Zn and monensin on ruminal degradation of Lys and liquid 2-hydroxy-4-methylthiobutanoic acid (HMB).

MATERIALS AND METHODS

Four nonlactating, mature, Holstein cows (mean BW 613.7 ± 11.9 kg) were surgically fitted with ruminal cannulas. All surgeries and animal handling were completed under protocols approved by the LSU Agricultural Center's Institutional Animal Care and Use Committee. A Latin square design experiment was used with 14-d periods that included 13 d of dietary adjustment followed by 1 d of sampling. Cows were housed in individual concrete-floored pens of approximately 3×5 m. Cows had access to fresh water at all times. At the completion of each period, cows were allowed to exercise in a 2-ha lot overnight.

Cows were fed complete mixed diets at restricted amounts once daily at 0800 h. Diets consisted of 4.54 kg (as fed) of a commercial chopped alfalfa hay (Bert & Wetta Larned, Inc., Larned, KS) that also contained trace amounts of molasses, mineral oil, and propionic acid for dust control and storage stability and 4.54 kg (as fed) of an experimental concentrate daily (Table 1). Diets were offered in restricted amounts to ensure complete consumption in a short period of time. Formulated nutrient density of the diets was such that maintenance requirements for protein and energy (National Research Council, 2001) were met. Concentrates contained ZnSO₄ (to provide 500 mg/kg of Zn in the final diet) and sodium monensin (Elanco Animal Health, Inc., Indianapolis, IN; to provide 40 mg/kg of monensin in final diet) in a 2×2 factorial arrangement of treatments. Concentrates and alfalfa were sampled on the last day of each period and stored at room temperature until analyzed for DM, ash, N, minerals (AOAC, 1980), ADF, and NDF (Van Soest et al., 1991).

On d 14 of each period, samples of ruminal fluid and whole blood were collected from each cow immediately prior to feeding. Samples of ruminal fluid (approximately 40 mL) were collected and strained through 4 layers of cheesecloth. Ruminal contents were obtained from beneath the ruminal mat, directly below the fistula opening of each cow. Dual samples of whole blood (7 mL) were collected from a coccygeal vessel of each cow into sterile evacuated containers and preserved with sodium fluoride and potassium oxalate (Sherwood Medical, St. Louis, MO). After these samples were collected, cows were fed and dosed through the ruminal cannula with Lys, HMB, and Cr-EDTA (Uden et al., 1980). The Cr-EDTA (200 mL; 0.956 g Cr/mL), HMB

(50 g), and Lys (100 g of Lys-HCl) were mixed with distilled H₂O to a total volume of 500 mL and administered as a liquid poured onto the top of the rumen mat but not mechanically mixed with ruminal contents.

Every 30 min after dosing, for a total of 8 h, samples (approximately 40 mL) of ruminal fluid were obtained by the same method as samples collected immediately prior to feeding. For all samples of ruminal fluid, pH was immediately measured, and the sample was frozen in liquid N then stored frozen (−20°C) until analyzed. Every 2 h after dosing, for a total of 8 h, dual samples of whole blood were collected, as described previously. Plasma was isolated after centrifugation at $2500 \times g$ for 15 min. One plasma sample was decanted into individual tubes, whereas the other was decanted into larger tubes for pooling across all time points. All plasma was stored frozen (−20°C) until later analysis.

All samples of ruminal fluid were allowed to thaw at room temperature and processed. A 4-mL aliquot of ruminal fluid was combined with 1 mL of 25% (wt/vol) meta-phosphoric acid that contained 2 g/L of 2-ethyl butyric acid. These samples were then centrifuged at $30,000 \times g$ for 25 min and the supernatant decanted and stored for VFA analysis (Bateman et al., 2002). The remainder of the ruminal fluid was clarified by centrifugation at $30,000 \times g$ for 25 min. Clarified ruminal fluid was separated into 2 subsamples and further processed prior to storage for analysis. The first sample (12 mL) was acidified by the addition of 1 mL of 20% (vol/vol) H₃PO₄ and stored until analysis for NH₄⁺ (Broderick and Kang, 1980), Cr (Williams et al., 1962), free Lys, and HMB. The analyses of Lys and HMB were conducted by the Central Analytical Laboratory, Center of Excellence for Poultry Science (University of Arkansas, Fayetteville). The second sample was prepared for peptide analysis by the addition of 200 μ L of saturated HgCl₂ solution to 4 mL of clarified ruminal fluid and then centrifuged at $10,000 \times g$ for 10 min (Fu et al., 2001). These samples were then subdivided further into 2 equal portions. One subsample was incubated in an equal volume of 70% HCl at 105°C for 24 h to hydrolyze peptides to their constituent AA. The concentration of ninhydrin-reactive material in both the hydrolyzed and unhydrolyzed samples was measured using Lys as a standard (Moore and Stein, 1954). Differences in ninhydrin-reactive material concentrations between the hydrolyzed and unhydrolyzed samples were considered peptide AA and were expressed as mM equivalent of Lys.

Over the last 24 h of each period, in situ disappearance of soybean meal and extruded soybean meal (West Central Soy, Inc., Ralston, IA) was measured. Samples (approximately 2 g as is) of each feed were weighed into 5 $\times$ 10 cm nylon bags with 50- μ m pore size (Amkon

Technology, Fairport, NY) and heat-sealed. Additional control blank bags were prepared that contained approximately 0.5 g of nylon cord. Duplicate samples of each feed and the nylon cord were incubated in the rumen of each cow over the last 24 h of each period for varying times. Bags were incubated for 0, 2, 4, 8, 16, or 24 h. Bags were inserted into the rumen in reverse order and removed simultaneously. After bags were removed, gross contamination was removed by washing in a stream of cold water. After all gross contamination was removed, individual bags were washed by hand in cool water until the effluent ran clear. Excess water was then removed from bags by hand, and the bags were dried at 55°C for 48 h. Bags that were incubated for 0 h were not inserted into the rumen of cows but did undergo the washing process. Bags with only nylon cord inside were evaluated to determine whether there was any contamination present that was not apparent to the eye. In no instance was there any appreciable gain in weight to these bags following incubation, so it was concluded that the washing procedure was successful at removing contamination.

The natural logarithm of the concentration of marker in ruminal fluid was regressed against time to estimate fluid turnover rate of the rumen (K_p) from the slope of this line. The natural logarithm of the percentages of the initial dose of Lys and HMB that remained at each time point were regressed against time to estimate the rate of disappearance (K_d) of these compounds (% of dose) from the ruminal environment from the slopes of these lines. These 2 values were combined within a cow and period using the following formula to estimate ruminal degradation of these compounds: % degradation = $K_d \div (K_d + K_p)$. Rate of disappearance of soybean meal and extruded soybean meal was estimated by regression of the natural logarithm of the concentration (% of original feed) remaining at each time point against time. Slopes of the resulting lines were considered to estimate ruminal degradation of the feeds.

When there was only one measurement per period, those data were analyzed as a Latin square-designed experiment using a mixed model that included terms for cow, period, level of Zn, level of monensin, and the interaction of Zn and monensin (Snedecor and Cochran, 1989). Cow nested within the interaction of period, Zn, and monensin was included as a random term that was used to test the main effects of Zn, monensin, and their interaction (Littell et al., 1998). Time series data were analyzed as repeated measures nested within the Latin square using a mixed model (Littell et al., 1998). The model included terms for cow, period, time, and treatment, and all interactions among time and treatment. Cow nested within period $\times$ Zn $\times$ monensin was included as a random term to test treatment main effects and

Table 2. Chemical analysis of concentrate mixtures and alfalfa hay fed to cows.

	– Zn		+ Zn		Alfalfa
	– Monensin	+ Monensin	– Monensin	+ Monensin	
DM, %	90.00	90.12	90.40	88.35	89.20
	% of DM				
Ash	5.18	4.92	4.59	5.12	10.37
N	3.488	3.518	3.016	3.333	2.923
NDF	11.43	12.24	10.96	12.39	41.29
ADF	3.94	4.02	4.79	4.49	34.31
Ca	0.45	0.34	0.40	0.35	1.26
Mg	0.17	0.17	0.17	0.19	0.20
K	0.92	0.84	0.77	0.80	2.08
	mg/kg				
Cu	33	11	23	14	5
Mn	50	50	68	67	33
Zn	113	101	644	588	17

their interactions. All data are presented as least square means. All calculations were completed using SAS (SAS Institute Inc., 1990).

RESULTS AND DISCUSSION

All concentrates were similar in percentages of DM, N, ADF, NDF, and ash (Table 2). Cows usually consumed their allotment of diets by 1000 h. If a cow had not consumed her ration by 1000 h on the day of sampling, the portion of the diet remaining was force-fed through the ruminal cannula.

Mean ruminal pH remained above 5.5 and was not affected by treatment (Table 3). There were no time-dependent changes in ruminal pH due to treatment. Mean ruminal concentrations of NH_4^+ were not affected by treatment. The postprandial pattern of ruminal NH_4^+ release or production was not affected by treat-

ment. Average ruminal concentrations of NH_4^+ were above 10 mg/dL at all sampling times. This is above the reported minimum for optimal ruminal fermentation (Grigsby et al., 1993), so it is unlikely that fermentation was impaired due to lack of available N. Ruminal concentrations of peptides averaged 15.0 mM and were not affected by treatments. This is well above the concentration of peptides suggested optimum for ruminal fermentation (Fu et al., 2001). Ruminal bacteria have been shown to increase their growth rate when supplied with sources of amino N, such as AA or peptides (Maeng et al., 1976) or when supplemented with HMB (Vazquez-Anon et al., 2001).

Mean concentrations of total VFA (Table 3) were not affected by the addition of monensin or Zn to diets ($P > 0.1$). Also there were no interactions ($P > 0.1$) of Zn or monensin and time postfeeding for concentrations of total VFA in ruminal fluid. There was a tendency ($P =$

Table 3. Least square means for ruminal parameters of cows fed diets with or without 500 mg/kg of supplemental Zn and with or without 40 mg/kg of monensin.

	– Zn		+ Zn		SEM
	– Monensin	+ Monensin	– Monensin	+ Monensin	
pH	5.81	5.72	5.85	5.92	0.10
Peptides, mM	17.3	20.0	12.6	9.9	5.0
NH_4^+ , mg/dL	21.2	20.0	22.0	21.1	2.98
Total VFA, mM	133.63	138.36	128.89	115.07	9.97
Acetate, mol % ¹	59.14	54.35	57.98	56.93	1.22
Propionate, mol % ¹	17.07	20.55	18.15	20.24	0.98
Isobutyrate, mol %	1.49	1.59	1.47	1.51	0.14
Butyrate, mol %	15.09	15.19	15.35	13.76	0.66
Isovalerate, mol % ²	3.50	4.18	3.44	4.08	0.34
Valerate, mol % ³	3.73	4.14	3.61	3.48	0.21
Ratio ^{1,4}	3.54	2.80	3.34	2.88	0.15

¹Effect of monensin, $P < 0.05$.

²Effect of Zn, $P \leq 0.1$.

³Effect of monensin, $P < 0.01$.

⁴Ratio of acetate to propionate in ruminal fluid.

Figure 1. Least square means for ruminal acetate concentrations of cows fed 0 (♦) or 500 (■) mg/kg of supplemental Zn.

0.09) for Zn to impact the proportion of acetic acid in ruminal fluid post feeding (Figure 1). Increased Zn in the diet resulted in an increase ($P = 0.04$) in the proportion of propionic acid in ruminal fluid after feeding (Figure 2). Neither Zn nor monensin affected the proportion of butyrate in ruminal fluid ($P > 0.1$). Increased Zn in the diet tended ($P = 0.1$) to decrease the proportion of valeric acid (Table 3). High levels of Zn altered ruminal concentrations of VFA (Froetschel et al., 1990; Arelovich et al., 2000; Bateman et al., 2002). The addition of monensin to diets decreased ($P = 0.05$) the proportion of acetic acid and increased the proportion of propionate ($P = 0.02$). Therefore, monensin decreased ($P = 0.003$)

Figure 2. Least square means for ruminal propionate concentrations of cows fed 0 (♦) or 500 (■) mg/kg of supplemental Zn.

the ratio of acetic to propionic acid. Inclusion of monensin in the diet tended ($P = 0.09$) to increase the proportion of isovaleric acid. Because there were no changes in total VFA concentrations, the shift in the ratio of acetic to propionic acid indicates that the addition of monensin to the diets improved the fermentation efficiency by capturing more of the gross energy from the feed as VFA.

Fractional rate of disappearance of soybean meal (SBM) DM from in situ bags increased ($P = 0.02$), and rate of disappearance of extruded SBM tended ($P = 0.08$) to be increased by Zn in diets (Table 4). This was unexpected and is in contrast to data reported by Froetschel et al. (1990), who report that supplemental Zn decreased fermentation of AA in the rumen. Differences between our results and those of Froetschel et al. (1990) may be related to differences in basal diet quality or supplementation levels. Bateman et al. (2002) reported that the effects of Zn on ruminal fermentation and urea degradation were different when supplemented with alfalfa, compared with low-quality forages. This may be related to differences in dietary protein content. Diets fed by Froetschel et al. (1990) were 16% CP or lower, but our diets were approximately 19 to 20% CP. Additionally, monensin reduced ruminal Zn concentrations (Kirk et al., 1985). Our supplementation levels for Zn were much lower than those used by Froetschel et al. (1990) and may have been further reduced through the interaction of the Zn and the monensin in the rumen. Monensin did not affect the fractional rate of disappearance of SBM or extruded SBM ($P > 0.1$). There were no interactions of monensin and Zn in diets to alter the rate of disappearance of SBM or extruded SBM from in situ bags ($P > 0.1$). Fractional rate of disappearance of CP in SBM from in situ bags tended ($P = 0.07$) to increase when Zn was added to diets. Monensin and the interaction of monensin and Zn did not affect the fractional rate of disappearance of CP in SBM ($P > 0.1$). The fractional rate of disappearance of CP in extruded SBM was not affected by Zn, monensin, or their interaction ($P > 0.1$). Monensin decreases protein degradation in the rumen (Bergen and Bates, 1984), but its impact on DM degradability is variable and seems dependent on the basal diet. It is possible that the lack of impact of monensin on CP degradability in this experiment was due to the lack of effects on DM degradability.

Supplemental Zn tended ($P = 0.06$; Table 4) to increase the fluid passage rate from the rumen. Supplemental monensin increased ($P = 0.02$) the ruminal fluid passage rate. Increased ruminal turnover rate may improve microbial efficiency (Demeyer and Van Nevel, 1986; Baumont and Deswysen, 1991) due to faster washout of the microbes from the rumen. If there is

Table 4. Least square means for fractional disappearance rates of from the rumen of cows fed diets with or without 500 mg/kg of supplemental Zn and with or without 40 mg/kg of monensin.

	– Zn		+ Zn		SEM
	– Monensin	+ Monensin	– Monensin	+ Monensin	
SBM DM K_d , %/h ¹	3.2	4.4	5.1	6.2	0.69
SBM CP K_d , %/h ²	3.6	5.7	6.2	7.0	0.92
Ex. SBM DM K_d , %/h ²	2.0	2.2	2.9	3.2	0.49
Ex. SBM CP K_d , %/h	3.8	4.3	5.1	6.1	0.90
Fluid Passage, %/hr ^{2,3}	10.1	14.5	13.7	16.0	1.08
Lysine K_d , %/h	15.8	11.5	14.6	17.6	3.19
Lysine degradation, % ⁴	61.2	44.2	47.6	51.6	5.33
HMB K_d , %/h	15.3	19.7	19.4	17.8	3.53
HMB degradation, %	60.7	57.1	57.4	50.9	4.93

¹Effect of Zn, $P < 0.05$.²Effect of Zn, $P < 0.1$.³Effect of monensin, $P < 0.05$.⁴Interaction of Zn and monensin, $P < 0.1$.

an increase in microbial efficiency due to added Zn or monensin, the associated increase in microbial AA flow at the duodenum should improve the protein status of the host animal.

Ruminal concentrations of Lys and HMB were evaluated for outliers prior to analysis (Graybill and Iyer, 1994). One observation of ruminal concentration of free Lys was deleted prior to statistical analysis. The Studentized deleted residual of this data point indicated that it was an outlier. We believe that this one data point represents a measurement, whereas the added Lys did not have ample opportunity to mix throughout the rumen. There were no effects of Zn or monensin on the fractional disappearance of Lys or HMB from the rumen ($P > 0.1$). There was a tendency for an interaction ($P = 0.09$) of Zn and monensin on the ruminal degradability of Lys. The addition of monensin to low Zn diets tended to decrease the ruminal degradability, whereas the addition of monensin to high Zn diets tended to increase the ruminal degradability. Mean ruminal degradability of HMB was 56.5% and was not affected by the addition of Zn or monensin. Pearson correlation coefficients between the K_d for Lys and HMB and K_p were calculated. The K_d of Lys was negatively correlated ($r = -0.064$) with ruminal passage. Similarly, the K_d of HMB was negatively correlated ($r = -0.205$) with ruminal passage. However, neither of these correla-

tions was significant ($P > 0.1$). Although the correlations were not statistically significant, the negative relationship between K_d for Lys and HMB and K_p may explain the lack of effect of Zn and/or monensin on ruminal degradability of Lys and HMB.

Urea N in plasma averaged 12.96 mg/dL and was not affected ($P > 0.1$) by the addition of Zn or monensin to diets (Table 5). Peripheral plasma concentrations of Met averaged 27.3 nmol/mL. Lysine concentrations in peripheral plasma averaged 126.1 nmol/mL. Neither Met nor Lys concentrations in plasma were affected ($P > 0.1$) by the addition of Zn or monensin to diets. Our data suggest that there was a high level of escape of free AA from the rumen. We could not determine from our data whether this escape was due to flow or absorption across the rumen wall. In either event, there would seem to have been a high likelihood that concentrations of either Met or Lys could have increased in the blood rapidly after dosing. Unfortunately, rapid entry of these AA into the blood would not have permitted any opportunity to detect treatment differences in the pooled samples used in this experiment.

CONCLUSIONS

Feeding 500 mg/kg of Zn did not alter ruminal metabolism of HMB or Lys but tended to increase the rate of

Table 5. Least square means for plasma concentrations of urea N, Lys, and Met from cows fed diets with or without 500 mg/kg of supplemental Zn and with or without 40 mg/kg of monensin.

	– Zn		+ Zn		SEM
	– Monensin	+ Monensin	– Monensin	+ Monensin	
Urea N, mg/dL	13.57	13.85	12.12	12.54	1.00
Lys, nmol/mL	122.33	126.25	142.12	113.55	17.73
Met, nmol/mL	25.23	25.33	29.18	29.38	2.35

passage of fluid from the rumen. This increase in rate of passage may influence ruminal degradability of protein sources and microbial efficiency. Monensin and Zn interacted to alter ruminal degradability of Lys but not HMB. These data indicate that supplementing Zn greatly above requirements can be used to alter ruminal fermentation to capture increased feed energy as VFA. Additionally, these data reinforce the knowledge that providing monensin to ruminants alter ruminal fermentation and increase the energy capture from the diet while improving the protein status of the host animal.

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