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Hydrogen Sulfide Gas Levels Post Feeding

Sarah J. Vanness
University of Nebraska-Lincoln

Nathan Meyer
University of Nebraska-Lincoln, nmeyer2@unl.edu

Terry J. Klopfenstein
University of Nebraska-Lincoln, tklopfenstein1@unl.edu

Galen E. Erickson
University of Nebraska-Lincoln, gerickson4@unl.edu

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Summary

Dietary sulfur level is associated with hydrogen sulfide gas (H_2S) levels in the rumen. These studies quantified H_2S levels at different times post feeding with or without added iron (Fe) or copper (Cu) to bind sulfur. In addition, the correlations of ruminal pH measurements to ruminal H_2S gas levels were estimated. Correlations between ruminal pH and hydrogen sulfide levels were not large and Fe and Cu did not affect H_2S levels.

Introduction

Hydrogen sulfide (H_2S) gas is hypothesized to be associated with polioencephalomalacia (polio). In ruminants, sulfur compounds may bind copper (Cu) and iron (Fe) so they become unavailable for the animal. The objective of the current study was to feed Fe and Cu in excess of dietary requirements to bind to S and to prevent S from being metabolized into H_2S. Rumen gas collections at different times post feeding will inform us when H_2S levels peak.

Procedure

In Experiment 1, five ruminally fistulated steers were used in a 4 x 4 Latin square. Two steers were on the same diet throughout the trial. Treatments were as follows: 1) no added mineral; 2) 1500 ppm iron and 100 ppm copper; 3) 3000 ppm iron and 200 ppm copper; and 4) 4500 ppm iron and 300 ppm copper. All animals were fed the same base diet with corresponding treatment supplements. The base diet included 50% wet distillers grains plus solubles (WDGS), 19.5% dry-rolled corn (DRC), 19.5% high-moisture corn (HMC), 6% cornstalks and 5% supplement (DM basis). The base diet had a sulfur content of 0.53%.

Ten-day periods were used with eight days of adaptation and two days of collection. Cattle were housed in individual pens and fed once daily at 0800. Feed refusals were collected and weighed if present. Each individual bunk was suspended from a load cell, bunk weights were collected every minute and meal characteristics were calculated (Table 2).

Gas collection devices were inserted through the ruminal cannula into the rumen on day 9 prior to feeding. Ruminal gas samples were collected at 0, 4, 8 and 12 hours post feeding. Once the gas sample was collected, it was injected into water. Two reagents that react with H_2S were added to these water solutions, creating a blue color that has a wavelength of 670 nm. Samples were plated in a 96-well plate and read on a spectrophotometer at 670 nm. This procedure is similar to a photometric procedure determined by Kung Jr. et al. (*Journal of Dairy Science 81*:2251).

In Experiment 2, nine ruminally fistulated steers were used in a switch back design. The experiment was designed to evaluate a direct-fed microbial (DFM) on the incidence of acidosis as reported by Rolfe et al. (*2009 Nebraska Beef Report* pp. 99-101). The objective of the current experiment was to quantify the amount of H_2S produced at different times post feeding and determine correlations between ruminal pH and H_2S levels. Intake data were collected as in Experiment 1. Wireless pH probes were inserted into the steers to record ruminal pH every minute. The rumen gas cap was sampled for H_2S on the last day of each step during the step-up phase and every seven days while the animals were on the concentrate diet. Samples were taken at 6 and 12 hours post feeding.

For the step-up phase, steers were stepped up onto a finisher with four steps by removing alfalfa and increasing the level of high moisture corn (HMC) in the diet. The final finishing diet contained 57.5% HMC, 30% WDGS, 7.5% alfalfa and 5% supplement on a DM basis (Table 1). The S level of this diet was 0.34%. No additives were used to prevent sulfur from metabolizing in the rumen for this trial. Gas samples were analyzed as described for Experiment 1.

For Experiment 1, data were analyzed using the MIXED procedure of SAS (SAS Inst Inc.). Treatment was included in the model as a fixed effect, with animal being the random effect. No day x treatment interactions were observed ($P > 0.16$); therefore, only main effects of treatment and time are presented.

For Experiment 2, correlation procedure of SAS was used to determine correlations between pH and H_2S values. With the high variability in individual data, correlations were not strong.

Results

In Experiment 1, no significant differences were present among treatments for average meal size, number of meals or average meal length (Table 2). There was a tendency for cattle fed 4500 ppm Fe and 300 ppm Cu to spend less total time eating.

Table 1. Composition of adaptation diets in Experiment 2.

Days	1-7	8-14	9-21	22-28	29-120
Ingredient % DM	Step1	Step 2	Step 3	Step 4	Finisher
WDGS[1]	30.00	30.00	30.00	30.00	30.00
HMC[2]	20.00	30.00	40.00	50.00	57.50
Alfalfa	45.00	35.00	25.00	15.00	7.50
Supplement[3]	5.00	5.00	5.00	5.00	5.00
Dietary sulfur	0.40	0.38	0.37	0.35	0.34

[1]WDGS = wet distillers grains plus solubles.
[2]HMC = high moisture corn.
[3]Supplement contains 65.3% fine ground corn and 27.4% limestone.

Table 2. Experiment 1 meal characteristics[1] and intake for each level of added iron/copper.

Item	Control	Treatment 1500/100 Fe:Cu	Treatment 3000/200 Fe:Cu	Treatment 4500/300 Fe:Cu	SE	*P*-value
DMI	27.6	26.6	26.4	27.1	0.4	0.05
Number of meals, n	5.8	5.7	5.3	4.7	0.4	0.26
Avg. size, lb	9.5	8.1	9.2	11.0	1.2	0.44
Avg. length, min	125.3	118.8	124.9	128.7	13.1	0.96
Total length, min	607.6	608.2	635.9	541.4	22.5	0.08

[1]Meal characteristics include number of meals consumed per day, lb of feed consumed per meal, and average and total length of meals in minutes.

Table 3. H_2S levels at different hours post feeding for each of the added iron and copper levels in Experiment 1.

Item	Control	Treatment 1500/100 Fe:Cu	Treatment 3000/200 Fe:Cu	Treatment 4500/300 Fe:Cu	SE	*P*-value
0[1]	3.3	1.5	1.9	2.3	1.0	0.59
4	15.1	19.6	16.4	17.1	5.9	0.96
8	15.7	19.3	13.0	15.1	5.7	0.89
12	20.6	22.8	13.2	20.7	4.5	0.46

[1]H_2S values are expressed as µmol of H_2S per mL of rumen gas collected at 0-12 hours.

Table 4. Average ruminal H_2S[1] concentrations for grain adaptation and finishing diet in Experiment 2.

Diets[2]:	1	2	3	4	Finishing
6 h H_2S	4.60	9.11	10.65	8.92	9.41
12 h H_2S	3.89	6.83	9.08	14.44	16.61

[1]H_2S levels are reported as µmol H_2S per mL of rumen gas collected.
[2]All diets contained 30% wet distillers grains plus solubles and 5% supplement. As cattle were adapted to the finishing diet, the amount of alfalfa hay included decreased from 45 to 35 to 25, 15 and finally 7.5% as the cattle adapted from diets 1, 2, 3, 4 and the finisher, respectively. For every decrease in alfalfa hay, a corresponding increase in HMC was observed.

Table 5. Correlation of pH to H_2S Levels Experiment 2.

Item	6 hour	*P*-value	12 hour	*P*-value
Step-up				
Area < 5.6[1]	0.11	0.54	-0.18	0.31
Previous day time < 5.6[2]	-0.05	0.77	-0.33	0.06
Time < 5.6[3]	-0.17	0.33	-0.36	0.04
Finisher				
Area <5.6	-0.10	0.35	-0.15	0.15
Previous day time <5.6	-0.03	0.74	-0.20	0.05
Time <5.6	-0.19	0.06	-0.25	0.01

[1]Area < 5.6 = Magnitude below pH 5.6 multiplied by minutes below pH 5.6.
[2]This value is the amount of time ruminal pH was below 5.6 one day prior to H_2S collection.
[3]Time < 5.6 = Total time ruminal pH was below 5.6.

Dry matter intake (DMI) was different ($P = 0.05$), with average intakes of 27.6, 26.6, 26.5 and 27.1 lb/day for control (0/0), 1500/100, 3000/200, and 4500/300 ppm Fe/Cu, respectively. No effects ($P > 0.05$) were observed for H_2S levels at 0, 4, 8 or 12 hours post feeding due to Fe and Cu addition (Table 3). A significant difference was seen among time points across all treatments with zero hours post feeding being significantly lower than the other time points ($P < 0.01$). There was no time x treatment interaction ($P = 0.93$). In Experiment 1, a H_2S level of 22.8µmol/mL was seen at 12 hours post feeding when dietary S was 0.53%.

During the step-up phase in Experiment 2, H_2S levels increased numerically as the cattle moved through the adaptation diets. During the step-up phase numerically higher levels of H_2S were seen at 6 hours than at 12 hours for adaptation diets 1-3, while 6-hour values were numerically lower than 12-hour values for the final adaptation diet and the finishing diet (Table 4).

In Experiment 2, the ruminal H_2S concentration was weakly correlated to ruminal pH (Table 5). In general, the 6-hour H_2S values had higher correlation coefficients than the 12-hour values. However all correlation coefficients were relatively low, probably due to high variability within individual H_2S values. Average H_2S levels for cattle at 6 and 12 hours post feeding were 9.01 and 13.7 µmol/mL of rumen gas collected, respectively, for the finishing diet that contained 0.34% S.

Based on the correlations, we conclude that ruminal pH is not a good indicator of increased H_2S production when levels of dietary sulfur are moderately high. At this time we do not have a clear answer as to whether increased H_2S levels are a result of increased dietary sulfur level or decreased ruminal pH.

[1]Sarah J. Vanness, research technician; Nathan F. Meyer, research technician; Terry J. Klopfenstein, professor; Galen E. Erickson, associate professor, Animal Science, Lincoln, Neb.