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Ruminal Sulfide Levels in Corn Byproduct Diets with Varying Roughage Levels

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Summary

Ruminally fistulated steers with wireless pH probes were utilized to quantify ruminal pH plus hydrogen sulfide (H₂S) levels produced at different times post feeding and to determine the effect of roughage level in high byproducts diets on hydrogen sulfide production. Because of variation in H₂S levels, ruminal pH was not related to high H₂S levels. When treatment means were used, pH and H₂S levels were highly correlated. We observed lower H₂S levels in diets with 7.5% or 15% grass hay compared with no roughage.

Introduction

In a recent finishing study (Wilken et al., 2009 *Nebraska Beef Report*, pp. 76-78), steers fed 66% wet distillers grains plus solubles (WDGS) with a higher roughage level (29.4% DM) did not experience polio (sulfur level 0.55%), whereas cattle fed a diet with a somewhat lower level of sulfur (0.48%) and low roughage (7.5% DM) did experience some polio cases. Based on a recent summary of University of Nebraska–Lincoln byproduct research (Vanness et al., 2009 *Nebraska Beef Report*, pp. 79-80), cattle can tolerate up to 0.46% sulfur with little risk (0.1%) of polio. The National Research Council (2003) suggests cattle fed corn-based diets can tolerate only 0.30% sulfur in the diet.

It is believed that hydrogen sulfide (H₂S) production by rumen microorganisms is the direct cause of polio-encephalomalacia with high dietary S levels. An objective of the current study was to determine the effect of roughage level in high byproduct

diets on H₂S levels in the rumen. An added objective was to determine the relationship between ruminal pH and hydrogen sulfide concentration.

Procedure

In Experiment 1, seven ruminally fistulated steers were fed during a 4-week adaptation period. Steers were housed in individual pens with bunks suspended from load cells. Cattle were fed twice daily at 0700 and 1600 with 50% of the feed at each time. Bunks were evaluated and residual feed weighed before the 0700 feeding. All steers were stepped up on the same diet. Each grain adaptation diet was fed for seven days with a common finisher being fed in week 4 (Table 1). Steers were fed decreasing amounts of alfalfa hay and increasing amounts of dry-rolled corn (DRC) for three weeks, with wet distillers grains plus solubles (WDGS) held constant at 50% diet DM. Wireless pH probes were inserted to measure ruminal pH. Measurements were taken every minute and recorded onto a data logger. Loggers were downloaded prior to feeding on the first day of each adaptation diet.

The finishing diet included 50% WDGS (received from Abengoa Bioenergy, York, Neb.), 37.5% DRC, 7.5% alfalfa and 5% supplement. The dietary S level was 0.44%.

Gas samples were collected on the last day of each step. Gas collection

devices were inserted through the ruminal cannula prior to feeding on day 7 and samples were collected at 1500 that day and 0600 (prior to feeding) on the next day (day 1 of the next adaptation diet). Four gas samples were taken from each steer at each time point.

In Experiment 2, seven ruminally fistulated steers were used in a 6 x 6 Latin square design. Two steers consumed the same diet throughout the trial. A 3 x 2 factorial treatment design was used. The first factor was three different inclusion levels of grass hay (0%, 7.5% or 15%, DM basis), while the second factor was two different byproduct inclusion levels and sources (Table 2). One of the diets tested by Wilken and others (2009 *Nebraska Beef Report*, pp. 76-78) consisted of a 50:50 blend of wet corn gluten feed (WCGF) and WDGS. That diet was replicated in this experiment. Each period was seven days (six days of adaptation and one day of collection).

Steers were housed in individual pens with bunks suspended from load cells. Bunk measurements were taken every minute. Steers were fed twice daily with equal amounts at 0700 and 1600. Feed amounts were determined and feed refusal weighed if present before the 0700 feeding. Rumen gas samples were collected on day 7 of each period as described above.

Data were analyzed as a 6 x 6 Latin square using the MIXED procedure

(Continued on next page)

Table 1. Diet compositions and nutrient analysis of adaptation diets in Experiment 1.

Items	Diet 1	Diet 2	Diet 3	Finisher
WDGS ¹	50.0	50.0	50.0	50.0
Alfalfa	35.0	25.0	15.0	7.5
DRC ²	10.0	20.0	30.0	37.5
Supplement	5.00	5.0	5.0	5.00
CP, % DM	24.96	23.89	22.82	22.02
Fat, % DM	6.66	6.90	7.13	7.31
NDF, % DM	29.48	27.32	25.16	23.54
Sulfur, % DM	0.50	0.48	0.46	0.44

¹WDGS = wet distillers grains plus solubles.

²DRC = dry-rolled corn.

Table 2. Diet compositions and nutrient analysis of byproduct combination diets with varying amounts of grass hay in Experiment 2.

Roughage level:	50% WDGS			37.5% WDGS /37.5%WCGF		
	0	7.5	15	0	7.5	15
WDGS ¹	50.0	50.0	50.0	37.5	37.5	37.5
WCGF ²	0.0	0.0	0.0	37.5	37.5	37.5
Grass hay	0.0	7.5	15.0	0.0	7.5	15.0
DRC ³	45.0	37.5	30.0	20.0	13.5	6.0
Supplement	5.0	5.0	5.0	5.0	5.0	5.0
Nutrient composition						
CP, % DM	21.9	21.8	21.7	25.4	25.3	25.2
NDF, % DM	21.8	26.3	30.8	28.4	32.9	37.4
Fat, % DM	7.2	7.2	7.1	6.2	6.2	6.1
Sulfur, % DM	0.43	0.42	0.41	0.47	0.46	0.45

¹WDGS = wet distillers grains plus solubles.

²WCGF = wet corn gluten feed.

³DRC = dry-rolled corn.

Table 3. Effects of adaptation diet on pH and H₂S values in Experiment 1.

	Diet 1	Diet 2	Diet 3	Finisher	P-Value
DMI, (lb/d)	14.70	16.70	19.49	20.62	< 0.01
Average pH	6.05	5.51	5.49	5.51	< 0.01
Max pH	6.65	6.27	6.08	6.19	< 0.01
Min pH	5.54	5.10	5.14	5.15	< 0.01
pH change	1.14	1.17	0.94	1.05	< 0.01
Area < 5.6 ¹	0.38	335.39	285.18	1438.79	< 0.01
Area < 5.3 ¹	6.51	150.95	91.07	73.09	0.02
H ₂ S 8 h ²	8.90	8.30	47.70	121.50	< 0.01
H ₂ S 23 h ³	6.20	4.50	21.20	33.30	0.05

¹Area is magnitude of pH under respective pH by minute.

²H₂S values are μmol hydrogen sulfide gas per mL of rumen gas collected 8 hours post feeding.

³H₂S values are μmol hydrogen sulfide gas per mL of rumen gas collected 23 hours post feeding.

Table 4. Main effects of byproduct for intake, ruminal pH and H₂S in Experiment 2.

Item	WDGS	WDGS/WCGF ¹	SE	P-value
DMI (lb/day)	20.6	21.1	0.3	0.15
Average pH	5.69	5.87	0.0	<0.01
Max. pH	6.31	6.47	0.0	<0.01
Min. pH	5.26	5.45	0.0	<0.01
Area < 5.6 ²	253.5	168.2	60.5	0.26
pH change	1.04	1.01	0.0	0.71
pH variance	0.06	0.05	0.0	0.20
H ₂ S 8 h ³	53.1	87.9	15.7	0.13
H ₂ S 23 h ⁴	88.2	65.0	20.0	0.28

¹WDGS = wet distillers grains plus solubles, WCGF = wet corn gluten feed.

²Area under curve is magnitude of pH < 5.6 by minute.

³Values are μmol hydrogen sulfide/mL rumen gas collected 8 hours post feeding.

⁴Values are μmol hydrogen sulfide/mL rumen gas collected 23 hours post feeding.

of SAS (SAS Inst. Inc.). Treatment was included in the model as a fixed effect with animal being the random effect. No byproduct x grass hay level interactions were observed ($P > 0.23$); therefore, only main effects of byproduct or grass hay levels were reported. The correlation procedure of SAS was used to determine correlations between pH and H₂S values. With the high

variability in individual data, treatment means also were used for correlation calculations.

Results

In Experiment 1, H₂S levels increased as roughage decreased (grain adaptation) at both 8 and 23 hours post feeding, ($P < 0.01$

and $P = 0.05$, respectively; Table 3). We hypothesized that H₂S levels would increase as roughage level decreased during grain adaptation. The increased H₂S production could be a result of reduced dietary fiber or increased dietary starch concentration. Intake increased ($P < 0.01$) as the cattle were adapted over the 21 days prior to the finishing diet.

Average pH, maximum pH and minimum pH decreased ($P < 0.01$) as the cattle were stepped up to the finisher diet. The area under pH 5.6 and 5.3 increased ($P < 0.01$) as cattle were adapted to the finisher diet.

In Experiment 2, there were no byproduct x grass hay level interactions; therefore, main effects are presented (Table 4). Cattle fed the combination byproduct diet had greater dry matter intake (DMI; $P = 0.07$); however, average, maximum and minimum ruminal pH levels also were higher ($P < 0.01$) than in those who received the diet with lower byproduct inclusion. The H₂S levels were not different between the two diets. No differences were observed between byproduct diets for area under pH 5.6, pH change (maximum-minimum), or pH variance ($P > 0.10$).

With increasing grass hay levels in the diets (Table 5), DMI and average, maximum and minimum ruminal pH increased linearly ($P < 0.03$). No differences were observed for area under pH 5.6, 5.3 or 5.0. At 8 hours post-feeding, H₂S levels declined linearly as grass hay levels in the diets increased ($P < 0.01$). At 23 hours post feeding, a numerical decrease in H₂S was observed with increasing grass hay levels. Because of the relatively high ruminal pH levels with the combination byproduct diet, it might be tempting to remove the roughage from the diet to improve feed efficiency. These data illustrate that the H₂S level of the diet with 7.5% hay was 44% of the H₂S level in the no roughage diet. Therefore, the risk of polio is expected to be much greater for cattle fed the no roughage diet; diets should contain at least 6-7% roughage.

The 7.5% hay diet is probably typical of most commercial feedlot diets.

Doubling the hay level to 15% reduced H₂S levels in the rumen. Approximately 55% less H₂S was produced in the 15% hay diet compared to the 7.5% hay diet at 8 hours post feeding.

At this time, we have not developed a cause-and-effect relationship between ruminal H₂S levels and polio. However, we assume the risk of polio is decreased if ruminal H₂S levels are decreased. Feeding additional roughage with high byproduct diets appears to reduce H₂S levels and therefore the risk of polio.

We hypothesized pH to be positively correlated with the level of H₂S concentration in the rumen. There were no significant correlations using individual animal data at 8 or 23 hours post feeding in either experiment (Table 6). The lack of significant correlations appears to be due to the large variability in H₂S concentrations; therefore, treatment mean correlations were calculated. In Experiment 1, area below pH 5.6 on the same day was correlated to H₂S levels at both 8 ($r = 0.94, P = 0.06$) and 23 hours ($r = 0.85, P = 0.15$) post feeding. In Experiment 2, there was a tendency for the 23-hour H₂S level to increase as average pH decreased ($r = -0.92, P = 0.13$). There also was a tendency for the 8-hour H₂S level to increase as the amount of time below pH 5.6 the previous day increased ($r = 0.98, P = 0.12$). At 23 hours post feeding, H₂S levels increased as the area below pH 5.6 of the same day increased ($r = 0.98, P = 0.13$). We conclude that average ruminal pH is negatively correlated with ruminal H₂S levels. Roughage level in the diet appears to be very important. In these experiments, dietary sulfur levels ranged from 0.47% to 0.41%; the H₂S levels ranged from 125.9 to 29.7 μmol/mL of rumen gas. In another study (Vanness et al. 2009 *Nebraska Beef Report* pp. 84-85), H₂S levels at 12

Table 5. Main effect of grass hay level in Experiment 2 for intake, ruminal pH and H₂S.

Item	Grass hay level			SE	Lin.	Quad.
	0	7.5	15.0			
DMI, lb/day	20.6	20.4	21.5	0.3	< 0.03	0.08
Average pH	5.62	5.75	5.96	0.0	< 0.01	0.42
Max pH	6.22	6.32	6.62	0.0	< 0.01	0.03
Min pH	5.25	5.37	5.44	0.0	< 0.01	0.58
Area < 5.6 ¹	306.0	176.8	149.7	72.0	0.08	0.54
Area < 5.31	73.4	29.6	39.2	20.0	0.19	0.27
Area < 5.0 ¹	4.3	2.4	2.4	1.6	0.33	0.59
pH change	1.0	1.0	1.2	0.0	< 0.01	< 0.01
H ₂ S 8 h ²	125.9	55.9	29.7	19.0	< 0.01	0.37
H ₂ S 23 h ³	91.5	84.1	54.2	30.0	0.45	0.67

¹Area is magnitude of pH under respective pH by minute.

²Values are μmol hydrogen sulfide mL of rumen gas collected 8 hours post feeding.

³Values are μmol of hydrogen sulfide mL of rumen gas collected 23 hours after first feeding.

Table 6. Correlation of ruminal pH to H₂S levels at 8 and 23 hours post feeding.

Item	8 hour	P-value	23 hour	P-value
Experiment 1				
Individual ¹				
Average pH	0.12	0.95	-0.05	0.79
Area < 5.6	-0.21	0.29	-0.03	0.86
Previous day time < 5.6 ²	-0.05	0.80	0.06	0.73
Time < 5.6	-0.01	0.95	0.01	0.95
Treatment mean ³				
Average pH	-0.47	0.53	-0.50	0.50
Area < 5.6	0.94	0.06	0.85	0.15
Previous day time < 5.6	0.37	0.63	0.42	0.58
Time < 5.6	0.45	0.55	0.50	0.50
Experiment 2				
Individual ¹				
Average pH	-0.05	0.76	-0.16	0.41
Area < 5.6	-0.06	0.70	0.02	0.90
Previous day time < 5.6	-0.00	0.98	0.30	0.11
Time < 5.6	-0.05	0.73	0.23	0.23
Treatment mean ²				
Average pH	-0.92	0.25	-0.98	0.13
Area < 5.6	0.99	0.07	0.77	0.44
Previous day time < 5.6	0.98	0.12	0.92	0.26
Time < 5.6	0.93	0.25	0.98	0.13

¹Correlations based on individual animal values for pH and H₂S.

²This value is the amount of time ruminal pH was below 5.6 one day prior to H₂S collection.

³Correlations based on treatment mean.

hours post feeding ranged from 19.3 μmol/mL when dietary S levels were 0.53%, to 13.7 μmol/mL when dietary S levels were 0.34%. This does not show a clear relationship between dietary S levels and ruminal H₂S levels. However, this comparison is across experiments and a conclusion

cannot be drawn until dietary S levels are compared within an experiment.

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