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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 ($H_2S$) 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 $H_2S$ levels, ruminal pH was not related to high $H_2S$ levels. When treatment means were used, pH and $H_2S$ levels were highly correlated. We observed lower $H_2S$ 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 ($H_2S$) production by rumen microorganisms is the direct cause of polioencephalomalacia with high dietary S levels. An objective of the current study was to determine the effect of roughage level in high byproduct diets on $H_2S$ 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 |

[1]WDGS = wet distillers grains plus solubles.
[2]DRC = dry-rolled corn.

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

| | 50% WDGS | | | 37.5% WDGS /37.5%WCGF | | |
|---|---|---|---|---|---|---|
| Roughage level: | 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 |

[1]WDGS = wet distillers grains plus solubles.
[2]WCGF = wet corn gluten feed.
[3]DRC = dry-rolled corn.

**Table 3. Effects of adaptation diet on pH and $H_2S$ 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 |
| $H_2S$ 8 h[2] | 8.90 | 8.30 | 47.70 | 121.50 | < 0.01 |
| $H_2S$ 23 h[3] | 6.20 | 4.50 | 21.20 | 33.30 | 0.05 |

[1]Area is magnitude of pH under respective pH by minute.
[2]$H_2S$ values are µmol hydrogen sulfide gas per mL of rumen gas collected 8 hours post feeding.
[3]$H_2S$ 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 $H_2S$ 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 |
| $H_2S$ 8 h[3] | 53.1 | 87.9 | 15.7 | 0.13 |
| $H_2S$ 23 h[4] | 88.2 | 65.0 | 20.0 | 0.28 |

[1]WDGS = wet distillers grains plus solubles, WCGF = wet corn gluten feed.
[2]Area under curve is magnitude of pH < 5.6 by minute.
[3]Values are µmol hydrogen sulfide/mL rumen gas collected 8 hours post feeding.
[4]Values 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 $H_2S$ values. With the high variability in individual data, treatment means also were used for correlation calculations.

## Results

In Experiment 1, $H_2S$ 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 $H_2S$ levels would increase as roughage level decreased during grain adaptation. The increased $H_2S$ 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 $H_2S$ 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, $H_2S$ levels declined linearly as grass hay levels in the diets increased ($P < 0.01$). At 23 hours post feeding, a numerical decrease in $H_2S$ 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 $H_2S$ level of the diet with 7.5% hay was 44% of the $H_2S$ 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 $H_2S$ levels in the rumen. Approximately 55% less $H_2S$ 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 $H_2S$ levels and polio. However, we assume the risk of polio is decreased if ruminal $H_2S$ levels are decreased. Feeding additional roughage with high byproduct diets appears to reduce $H_2S$ levels and therefore the risk of polio.

We hypothesized pH to be positively correlated with the level of $H_2S$ 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 $H_2S$ concentrations; therefore, treatment mean correlations were calculated. In Experiment 1, area below pH 5.6 on the same day was correlated to $H_2S$ 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 $H_2S$ level to increase as average pH decreased (r = -0.92, $P = 0.13$). There also was a tendency for the 8-hour $H_2S$ 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, $H_2S$ 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 $H_2S$ 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 $H_2S$ levels ranged from 125.9 to 29.7µmol/mL of rumen gas. In another study (Vanness et al. *2009 Nebraska Beef Report* pp. 84-85), $H_2S$ levels at 12 hours post feeding ranged from 19.3µmol/mL when dietary S levels were 0.53%, to 13.7µmol/mL when dietary S levels were 0.34%. This does not show a clear relationship between dietary S levels and ruminal $H_2S$ levels. However, this comparison is across experiments and a conclusion cannot be drawn until dietary S levels are compared within an experiment.

**Table 5. Main effect of grass hay level in Experiment 2 for intake, ruminal pH and $H_2S$.**

| | Grass hay level | | | | | |
|---|---|---|---|---|---|---|
| Item | 0 | 7.5 | 15.0 | SE | Lin. | Quad. |
| 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 |
| $H_2S$ 8 h[2] | 125.9 | 55.9 | 29.7 | 19.0 | < 0.01 | 0.37 |
| $H_2S$ 23 h[3] | 91.5 | 84.1 | 54.2 | 30.0 | 0.45 | 0.67 |

[1]Area is magnitude of pH under respective pH by minute.
[2]Values are µmol hydrogen sulfide mL of rumen gas collected 8 hours post feeding.
[3]Values are µmol of hydrogen sulfide mL of rumen gas collected 23 hours after first feeding.

**Table 6. Correlation of ruminal pH to $H_2S$ 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 |

[1]Correlations based on individual animal values for pH and $H_2S$.
[2]This value is the amount of time ruminal pH was below 5.6 one day prior to $H_2S$ collection.
[3]Correlations based on treatment mean.

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