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Increasing dietary neutral detergent fiber concentration decreases ruminal hydrogen sulfide concentrations in steers fed high-sulfur diets based on ethanol coproducts

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ABSTRACT: Cattle feedlot diets commonly contain ethanol coproducts that are high in S. This dietary S is reduced in the rumen by sulfate reducing bacteria, resulting in an accumulation of hydrogen sulfide (H₂S), increasing the risk for S toxicity. A negative correlation between H₂S and ruminal pH has been observed previously. The objective of this study was to determine the effect of varying dietary NDF from chopped bromegrass hay (66% NDF) on performance, ruminal pH, and ruminal H₂S gas concentration of steers fed a high-S finishing diet. One hundred fifty crossbred steers (359 ± 51 kg BW) were blocked by BW into pens of 5 steers and randomly assigned within block to 1 of 5 treatments (*n* = 6 pens per treatment) and fed for 84 d. Dietary treatments included 3.5, 5.7, 7.9, 10.1, or 11.4% roughage NDF (rNDF) from bromegrass hay and contained 0.46% dietary S from a combination of dried distillers grains with solubles and condensed corn distillers solubles. In all diets, hay was added at the expense of dry-rolled corn. Effective NDF increased linearly (*P* < 0.01) with increased inclusion of rNDF. Final BW was not affected by rNDF (*P* ≥ 0.12). The addition of rough-

age did not affect ADG (*P* ≥ 0.13) or gain efficiency (*P* ≥ 0.12). Dry matter intake increased linearly (*P* < 0.01) as rNDF concentration increased. There was a treatment × month interaction for S intake (*P* < 0.01), explained by steers fed 3.5 or 11.4% rNDF increasing S intake each month whereas the middle rNDF inclusions had similar S intake between months 1 and 2 and increased in month 3. Ruminal H₂S concentrations and ruminal fluid pH were measured at 6 h postfeeding on d 7, 14, 21, 29, and 84. Ruminal pH increased linearly (*P* < 0.01; 5.48, 5.61, 5.71, 5.74, and 5.80 ± 0.041 for 3.5, 5.7, 7.9, 10.1, and 11.4% rNDF, respectively) and ruminal H₂S concentrations decreased linearly (*P* < 0.01; 1.00, 0.86, 0.76, 0.70, and 0.62 ± 0.037 g/m³ for 3.5, 5.7, 7.9, 10.1, and 11.4% rNDF, respectively) as rNDF inclusion increased. Using mixed model regression analysis, ruminal pH had a strong negative relationship with ruminal H₂S concentrations ($\beta = -0.63$; *P* < 0.01). Under conditions of this study, increasing roughage did not affect cattle gains but helped maintain greater ruminal pH and decreased H₂S concentration, suggesting that this dietary strategy may lessen the risk of S toxicity in feedlot cattle.

Key words: cattle, hydrogen sulfide, neutral detergent fiber, roughage, sulfur

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INTRODUCTION

Many cattle feeders utilize ethanol coproducts as protein and energy sources in feedlot cattle diets. Inclusion of ethanol coproducts is limited by high S content of these feedstuffs due to sulfuric acid use during the ethanol production process (Kwiatkowski et al., 2006). Increased dietary S has been shown to decrease

DMI and ADG (Richter et al., 2012) and increase the risk of S-induced polioencephalomalacia (PEM; Gould et al., 1997). Sulfur-induced PEM is thought to be the result of inhalation of eructated H₂S (Gould, 1998). At a more acidic pH, more sulfide is converted to H₂S in the rumen (Beauchamp et al., 1984). Previously, we reported that increasing from 4 to 7% roughage NDF (rNDF) from hay or cornstalks in a finishing diet containing 0.45% S increased ruminal pH and decreased H₂S concentrations, and pH and H₂S measurements were negatively correlated (Morine et al., 2014). This research supports the hypothesis that increasing rNDF modulates

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ruminal pH and lessens H₂S concentrations in cattle consuming high-S diets. Nichols et al. (2013) conducted a longitudinal analysis of data collected from studies that included cattle fed diets ranging from 0.12 to 0.73% S and a mean rNDF of 4% (DM basis) and compared rNDF concentration to the incidences of PEM. The authors suggested that when ruminally available S is accounted for, the prevalence of PEM decreased by 19% for every 1% of rNDF added to the diet. Therefore, the risk of S toxicity may be decreased by increasing rNDF in finishing diets with ethanol coproducts. However, dilution of dietary energy because of increased inclusion of roughage in place of high energy feedstuffs may have negative impacts on feedlot cattle growth and efficiency (Calderon Cortes and Zinn, 1996). Therefore, the objective of this study was to determine the impact of feeding various concentrations of rNDF from chopped bromegrass hay on performance, ruminal pH, and ruminal H₂S concentration of steers fed high-S diets based on ethanol coproducts.

MATERIALS AND METHODS

Procedures and protocols for this experiment were approved by the Iowa State University Institutional Animal Care and Use Committee (protocol 3-11-7146-B).

Animals and Experimental Design

One hundred fifty yearling Angus-type steers (359 ± 51 kg BW) were used to determine the effect of various rNDF concentrations in high-S finishing diets on ruminal pH, ruminal H₂S concentrations, and steer performance. Steers were transitioned to a high concentrate diet via 2 step up diets (Table 1) fed for 6 and 7 d, respectively. Once transitioned, consecutive 2-d weights were taken before feeding and steers were blocked by this pretrial BW to pens ($n = 5$ steers per pen) and within block pens were randomly assigned to receive 1 of 5 diets containing 3.5, 5.7, 7.9, 10.1, or 11.4% rNDF from bromegrass hay ($n = 6$ pens per treatment). The diets were analyzed to contain an average of 0.46% dietary S, which came primarily from a combination of dried distillers grains with solubles (DDGS) and condensed corn distillers solubles (CCDS). At the onset of the study, the treatment diets (Table 1) were limit fed for a 7-d period, with an initial feeding rate of 1.5% of BW daily, which then increased by 0.25% of BW daily until ad libitum intakes were reached. Consecutive 2-d weights were taken again at the end of the limit feeding period, which was considered d 0 and 1 of the trial.

Sample Collection and Analytical Procedures

Steers were fed once daily (0800 h) and bunks were managed to be slick at the time that feed calls were made (0630 h) as described by Drewnoski et al. (2014). The

Table 1. Ingredients and chemical composition of diets

Item, % DM	Step up 1	Step up 2	Roughage NDF, %				
			3.5	5.7	7.9	10.1	11.4
Chopped bromegrass hay ¹	30.0	17.3	5.3	8.6	11.9	15.3	17.3
DDGS ²	20.8	22.8	32.0	32.0	32.0	32.0	32.0
Soyhulls	14.0	12.5	—	—	—	—	—
CCDS ³	—	—	7.0	7.0	7.0	7.0	7.0
Dry-rolled corn	33.0	45.2	53.5	50.2	46.9	43.5	41.5
Limestone	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Salt	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Vitamin A premix ⁴	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Rumensin 90 ⁵	0.006	0.006	0.012	0.012	0.012	0.012	0.012
Trace mineral premix ⁶	0.035	0.035	0.035	0.035	0.035	0.035	0.035
Analyzed composition							
S, %	—	—	0.44	0.46	0.46	0.45	0.47
Calculated composition ⁷							
CP, %	—	—	14.7	14.8	14.8	14.8	14.8
NEm, Mcal/kg DM	—	—	2.01	1.98	1.94	1.90	1.87
NEg, Mcal/kg DM	—	—	1.41	1.38	1.34	1.31	1.29

¹Bromegrass hay (contained 66% NDF) was chopped using an 1150 Commercial Tub Grinder with Grapple Loader (Haybuster, Jamestown, ND) with a 15.24-cm screen.

²DDGS = dried distillers grains with solubles. Three loads of DDGS from POET (Jewell, IA) were used during the trial with S concentrations of 0.935, 0.87, and 0.86% (DM basis).

³CCDS = condensed corn distillers solubles. One load of CCDS from Rock-N-R Syrup Co. (Preston, MN) was used during the trial with S concentration of 1.28% (DM basis).

⁴Vitamin A premix contains 4,400,000 IU/kg.

⁵Targeted monensin at 200 mg·steer⁻¹·d⁻¹ (Elanco Animal Health, Greenfield, IN).

⁶Provided per kilogram of diet DM: 30 mg Zn as ZnSO₄, 20 mg Mn as MnSO₄, 0.5 mg I as Ca(IO₃)₂(H₂O), 0.1 mg Se as Na₂SeO₃, 10 mg Cu as CuSO₄, and 0.1 mg Co as CoCO₃.

⁷The calculated composition was determined based on analyzed content CP and Ohio Agricultural Research and Development Center energy calculation of ingredients.

amount of feed offered to each pen was recorded daily, individual ingredients and total mixed ration (TMR) samples were collected weekly for DM determination, and feed refusals for each pen were collected monthly for DM determination. Samples were dried in a forced-air oven at 70°C for 48 h. Monthly DMI was calculated on a pen basis by subtracting pen feed refusals from feed offered (DM basis). Sulfur analysis of TMR samples and pen feed refusals was conducted according to the method described by Richter et al. (2012) using an inductively coupled plasma–optical emission spectrometer (Optima 7000; PerkinElmer, Waltham, MA) and values were used to calculate dietary S intake. Samples of ingredients were taken at the beginning of the trial and sent to Dairyland Laboratories, Inc. (Arcadia, WI), for near infrared spectroscopy analysis. The analysis uses calibrated equations from these methods for NDF (AOAC, 2005 method 2002.04), nitrogen (AOAC, 1995 method 990.03), ether extract (AOAC, 1995 method 920.39),

and starch (Hall, 2009). Corn condensed distillers solubles were analyzed by wet chemistry (Dairyland Laboratories) using the methods above except ether extract was analyzed by acid hydrolysis using the Soxhapp 2047 and Soxtec extraction methods (Foss Analytical AB Soxtec System; Eden Prairie, MN). The NEg of each diet was then calculated based on the results of the Ohio Agriculture Research and Development Center energy calculation (Weiss et al., 1992). Steers were weighed on d 0, 1, 25, 56, 84, and 85 for determination of ADG and for calculation of feed efficiency.

Ruminal H₂S gas concentrations were measured at 6 h postfeeding from 2 steers per pen on d 7, 14, 21, 29, and 84. Sampling days were chosen because research has shown H₂S concentrations peak in approximately the first 30 to 60 d after cattle start consuming a high-S finishing diet (Drewnoski et al., 2012; Drewnoski and Hansen, 2013). Hydrogen sulfide concentration of the ruminal gas was measured by introducing a sterilized 16-gauge, 10.2-mm-long needle into the left paralumbar fossa. The needle was fitted with a short piece of tubing connecting the needle to a gas detector tube and volumetric gas sampling pump (Matheson-Kitagawa 8014-400B; Kitagawa, Kanagawa, Japan) according to the procedure described by Drewnoski et al. (2012). The equation used to convert H₂S concentration from parts per million to grams per cubic meter was $H_2S (g/m^3) = [(H_2S (ppm) \times 139.06)/1,000,000]$ assuming standard temperature and pressure values as described by Neville et al. (2010).

Ruminal fluid samples for determination of ruminal pH were taken at 6 h postfeeding from 1 of the 2 steers sampled for H₂S (a single steer was randomly selected on the first day and subsequently sampled for ruminal pH each time) in each pen on d 7, 14, 21, 29, and 84 immediately after H₂S concentrations were measured. The same sterilized 16-gauge, 10.2-mm-long needle that was introduced into the left paralumbar fossa for H₂S concentration determination was fitted to a 10-mL syringe, which was used to remove approximately 5 mL of ruminal fluid. The ruminal fluid was then transferred to a 15-mL conical tube and pH was immediately measured (Oakton pH 11 Meter Kit, Model 35614-80; Oakton Instruments, Vernon Hills, IL).

Neutral detergent fiber of the bromegrass hay and TMR samples were determined each month (d 8, 32, 63, and 81) using an ANKOM²⁰⁰ Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY) with the addition of α -amylase according to the procedures of Van Soest et al. (1991). Effective NDF (eNDF) was determined on samples collected on the same dates, using the Penn State Particle Size Separator as described by Kononoff et al. (2003). Particles greater than 7.87 mm were declared as eNDF.

Statistical Analysis

Data were analyzed by ANOVA as a randomized block design using the Mixed Procedure of SAS (SAS Inst. Inc., Cary, NC). The model for the analyses of NDF and eNDF included the fixed effect of treatment (concentration of rNDF) and the random effect of day. Final BW data were analyzed using a model containing the fixed effect of treatment and the random effect of block. Average daily gain, DMI, S intake, G:F, NEg intake, ADG to NEg intake (G:NEg), ruminal H₂S concentration, and ruminal pH data were analyzed as repeated measures and included the fixed effects of treatment, time, and the interaction between treatment and time. Block was considered a random effect. The repeated effect was day of sampling for ruminal pH and H₂S measures and month for ADG, DMI, S intake, G:F, NEg intake, and G:NEg. Selection of the best covariance structure was based on the lowest corrected Akaike's information criterion identified for the majority of the repeated measures models. Single df contrast statements testing the linear and quadratic effects of treatment (rNDF) were calculated using the orthopoly function of Proc IML. Pen was the experimental unit for all data analysis ($n = 6$ pens per treatment). Significance was declared at $P \leq 0.05$ and tendencies were declared at $0.05 < P \leq 0.10$. Outliers were identified for ADG, DMI, S intake, G:F, NEg intake, G:NEg, ruminal pH, and H₂S concentrations using the REG procedure of SAS, defined as data greater than 2 SD from the predicted mean, and less than 2% of the data were removed from the analysis. Means reported in the tables are least square means. A mixed model regression analysis fixed for treatment, day, and the interaction between ruminal pH and treatment, with ruminal pH as a covariate, the repeated effect of day, and random effect of block assessed the relationship between ruminal H₂S concentration and ruminal pH. The relationship between ruminal pH and H₂S is depicted using raw mean concentrations.

RESULTS

Diet NDF, Steer Intake, and Performance

The NDF and eNDF of total diets increased linearly with the increased inclusion of rNDF ($P < 0.01$; Table 2). Final BW was not affected by concentration of rNDF ($P \geq 0.12$; Table 3). There was no treatment \times month interaction for DMI ($P = 0.51$; data not shown). Dry matter intake increased linearly as rNDF increased ($P < 0.01$) and the data displayed a quadratic tendency ($P = 0.07$). The quadratic tendency reflects that intake was increased more by the 10.1 and 11.4% rNDF treatments than by the lesser additions of rNDF (3.5, 5.3, and 7.9%). For S intake there was an interaction between treatment and month ($P < 0.01$; Fig. 1). Steers fed the least and greatest

Table 2. Neutral detergent fiber and effective NDF (eNDF) concentrations of experimental diets

Item	Roughage NDF, %					SEM	P-value	
	3.5	5.7	7.9	10.1	11.4		Linear	Quadratic
NDF	21.2	23.8	25.4	26.9	29.5	0.96	<0.01	0.80
eNDF ¹	4.4	6.0	8.0	10.1	10.3	0.82	<0.01	0.72

¹Determined using Penn State particle size separator method described by Kononoff et al. (2003), declaring the NDF in particles greater than 7.87 mm as eNDF.

rNDF diets increased S intake each month, with the 3.5% rNDF treatment increasing most dramatically from month 2 to 3 whereas the middle inclusions of rNDF were similar across months 1 and 2 but increased in month 3.

The treatment × month interaction was not significant for any growth data ($P \geq 0.27$; data not shown). Concentration of rNDF did not affect ADG, G:F, or G:NEg ($P \geq 0.12$). There was a tendency for a quadratic effect of concentration of rNDF on NEg intake ($P = 0.09$). Dry matter intake and S intake steadily increased each month during the trial ($P < 0.01$). Average daily gain, G:F, NEg intake, and G:NEg were greatest during month 2 of the 3-mo trial, resulting in an effect of time ($P < 0.01$).

Ruminal pH

There was no treatment × time interaction for ruminal pH ($P = 0.41$; data not shown). Ruminal pH increased linearly as rNDF increased ($P < 0.01$; Fig. 2). Ruminal pH differed due to sampling day ($P < 0.01$), averaging 5.64, 5.59, 5.62, 5.59, and 5.90 ± 0.041 across all treatments on d 7, 14, 21, 29, and 84, respectively. The difference is driven by greater ruminal pH on d 84 compared with all other days.

Hydrogen Sulfide Measurements

Ruminal H₂S gas concentrations decreased linearly with the increasing concentration of rNDF ($P < 0.01$; Fig. 3). Ruminal H₂S concentrations also differed due to day ($P < 0.01$), averaging 0.79, 0.92, 0.85, 0.77, and 0.61 ± 0.037 g/m³ across all treatments on d 7, 14, 21, 29, and 84, respectively. The relationship between ruminal H₂S concentration and ruminal pH data is presented in Fig. 4.

Table 3. Effect of roughage NDF concentration on performance of finishing steers fed high-S diets

Item	Roughage NDF, %					SEM	P-value	
	3.5	5.7	7.9	10.1	11.4		Linear	Quadratic
Initial BW	362	360	357	359	357	9.3	0.70	0.90
Final BW	534	529	522	536	531	9.8	0.97	0.12
DMI, ¹ kg/d	10.7	10.7	10.8	11.3	11.3	0.18	<0.01	0.07
ADG, ¹ kg/d	2.04	1.97	1.94	2.07	2.04	0.053	0.55	0.13
G:F ¹	0.193	0.186	0.182	0.185	0.182	0.0052	0.12	0.38
NEg intake, ¹ Mcal/d	15.0	14.7	14.4	14.8	14.6	0.25	0.09	0.09
ADG:NEg intake ¹	0.137	0.135	0.135	0.142	0.141	0.0032	0.17	0.35

¹Repeated measures analysis: treatment × month ($P \geq 0.27$) and month ($P < 0.01$).

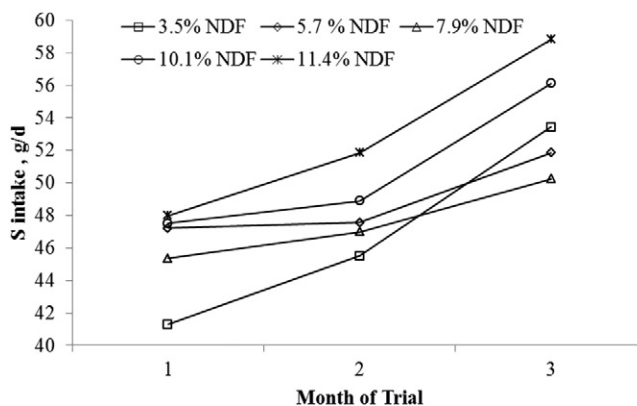


Figure 1. Sulfur intake of finishing steers fed various inclusions of roughage NDF (rNDF) 3.5 (□), 5.7 (◇), 7.9 (Δ), 10.1 (○), or 11.4% (×) rNDF as affected by rNDF concentration and month of study ($P < 0.01$). Effect of month ($P < 0.01$); SEM ± 1.19.

The regression of H₂S concentration and ruminal pH at 6 h postfeeding across d 7, 14, 21, 29, and 84 demonstrated a strong negative correlation ($\beta = -0.63$; $P < 0.01$).

DISCUSSION

The present study was designed to determine the impact of feeding various dietary concentrations of rNDF (3.5, 5.3, 7.9, 10.1, and 11.4%) from chopped bromegrass hay (66% NDF) on steer feedlot performance and ruminal pH and H₂S concentrations when fed a diet containing a moderate amount of S from ethanol coproducts. Ruminal pH and H₂S gas concentrations had a strong negative correlation. Previously, we reported that increasing rNDF (4, 7, or 10%) from either cornstalks or bromegrass hay decreased ruminal H₂S concentrations, and there was a strong negative correlation between ruminal pH and ruminal H₂S concentrations in steers fed 0.45% S, concentrate-based diets (Morine et al., 2014). Similarly, Vanness et al. (2009) reported when cattle were fed increasing amounts of grass hay (0, 7.5, or 15% diet DM) in coproduct diets averaging 0.44% dietary S there was a linear decrease in ruminal H₂S concentrations measured at 8 h postfeeding. These authors also reported that area below ruminal pH of

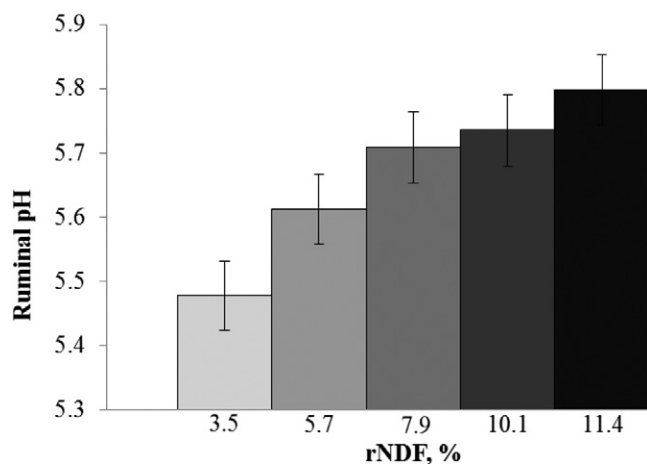


Figure 2. Effect of roughage NDF (rNDF) concentration on ruminal pH measured on d 7, 14, 21, 29, and 84 at 6 h postfeeding from finishing steers fed 3.5, 5.7, 7.9, 10.1, or 11.4% rNDF. Linear effect of rNDF ($P < 0.01$), quadratic effect of rNDF ($P = 0.27$), effect of day ($P < 0.01$), and rNDF concentration \times month interaction ($P = 0.41$).

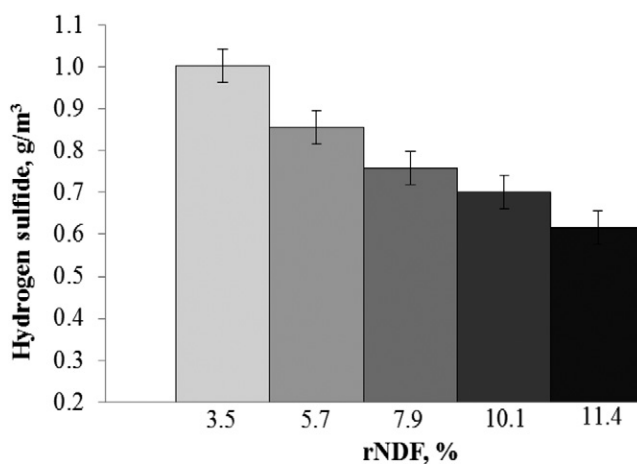


Figure 3. Effect of roughage NDF (rNDF) concentration on ruminal hydrogen sulfide concentration measured on d 7, 14, 21, 29, and 84 at 6 h postfeeding from finishing steers fed 3.5, 5.7, 7.9, 10.1, or 11.4% rNDF. Linear effect of rNDF ($P < 0.01$), quadratic effect of rNDF ($P = 0.45$), effect of day ($P < 0.01$), and rNDF concentration \times month interaction ($P = 0.67$).

5.6 tended to be positively correlated with H_2S concentrations measured 8 h postfeeding ($r = 0.99$, $P = 0.07$).

However, not all research has demonstrated a strong relationship between ruminal pH and H_2S gas concentrations. Sarturi et al. (2013) determined the effect of varying S sources on ruminal H_2S concentration of 5 ruminally cannulated beef steers. The authors calculated adjusted ruminal protein S (ARPS) by subtracting the calculated ruminally undegradable S from the total dietary S; ruminally undegradable S was estimated as the organic S from AA multiplied by the fraction of protein that was ruminally undegradable. By calculating ARPS, the authors predicted the ruminally available S, which is the S available to bacteria for reduction to sulfide. Consumption of ARPS and total dietary S varied across the treatments in this study, and the authors reported that average ruminal pH explained only 12% of the variation in ruminal H_2S concentrations, whereas ARPS intake explained 58% of the variation in ruminal H_2S concentrations (Sarturi et al., 2013). In the present study total dietary S, and likely ruminally available S content, was similar across treatments, and ruminal pH was strongly negatively correlated with H_2S concentrations. Intakes of ARPS by cattle will clearly affect ruminal H_2S concentrations; however, within a dietary concentration of ARPS ruminal pH appears to account for a large amount of the variation in ruminal H_2S .

Sulfate reducing bacteria are present in the rumen and utilize the dissimilatory sulfite reductase pathway to reduce dietary sulfate to sulfide, deriving energy from the process (Cummings et al., 1995). Given that the pK_a for H_2S is 7.04 for dissociation of the second ion (Beauchamp et al., 1984), approximately 97% of sulfide in the rumen should be found as H_2S at a pH of approximately 5.6. Previously, we reported that increasing rNDF from 4 to 7% decreased time under ruminal pH 5.6 and ruminal H_2S

concentrations (Morine et al., 2014). In the present study, ruminal pH linearly increased as dietary rNDF increased. The increase in ruminal pH from increased dietary roughage may in part be due to greater time spent chewing (Beauchemin and Yang, 2005). Increasing the time spent chewing results in secretion of more saliva, which contributes to the buffering capacity of the rumen (Selvaraj et al., 2007). Increased buffering capacity in steers fed greater rNDF may have modulated ruminal pH.

Nichols et al. (2013) analyzed data from cattle fed diets of 0.12 to 0.73% S and rNDF from 0 to 8% (DM basis) across several trials and noted that frequency of PEM appears to decrease 19% for every 1% increase in rNDF added to a diet when ruminally available S is accounted for in the model. Our preceding work (Morine et al., 2014) demonstrated that increasing rNDF in a high S, high concentrate diet slows the rate of DMI consumption and, in

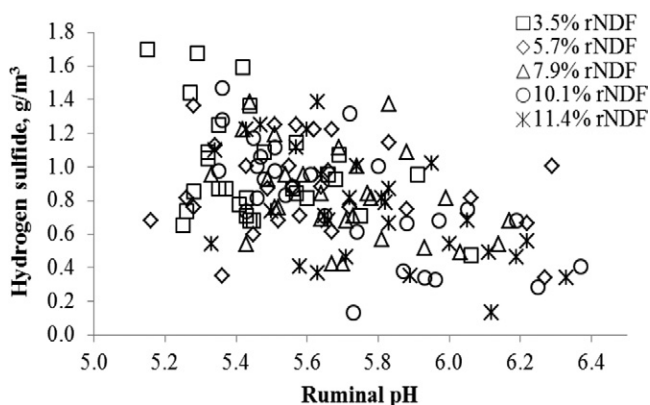


Figure 4. Mixed model regression analysis of ruminal hydrogen sulfide concentration and ruminal pH determined on d 7, 14, 21, 29, and 84 at 6 h postfeeding immediately after hydrogen sulfide measures were collected from finishing steers fed high-S diets and varying concentrations of roughage NDF: 3.5 (□), 5.7 (◇), 7.9 (△), 10.1 (○), or 11.4% (×) roughage NDF (rNDF; regression coefficient, $\beta = -0.63$; $P < 0.01$).

combination with the present study, suggests that increasing rNDF increases ruminal pH. Because the conversion of H₂S from sulfide is a pH-dependent process, the concentrations of H₂S gas in the ruminal head space decreases when ruminal pH increases. Hydrogen sulfide gas accumulation and subsequent inhalation has been proposed to be the cause of S toxicity in ruminants (Gould, 1998). The changes in cattle eating behavior and ruminal pH and their relationships to H₂S concentrations may help explain why increasing rNDF decreases risk of PEM in high-S diets.

Although the present study was not designed to induce PEM, it was designed to identify if increasing roughage in moderately high-S diets would decrease ruminal H₂S concentrations, which is the suspected cause of S-induced PEM. In our previous study (Morine et al., 2014) and the present study, no PEM cases were diagnosed and the average ruminal H₂S concentrations (0.48 g/m³ in Morine et al., 2014, and 0.77 g/m³ in the present study) suggest that tolerance to ruminal H₂S is greater than the previously reported threshold concentrations of 0.27 g/m³ for risk of PEM reported by Gould (1998), who utilized sodium sulfate to achieve PEM. Given the dietary S concentration in this study and the suggested incidence rate of PEM observed by Nichols et al. (2013), it is not unexpected that PEM was not observed in the present study. Additionally, inorganic S sources are assumed to be 100% ruminally available, whereas S AA are less available because the AA may bypass the rumen before being fully degraded. It is likely the S tolerance of cattle is greater when ethanol coproducts are included in the diet than when dietary S is provided to the diet from sodium sulfate, water sulfates, or other inorganic sources because the S would not be completely ruminally available when consumed in a coproduct form. The availability of dietary S for reduction in the rumen, the rate at which the S becomes available, ruminal pH, and likely other undetermined ruminal factors all influence ruminal H₂S gas concentrations.

An obvious negative outcome of increasing dietary rNDF concentration is the dilution of energy in the diet and the potential of this dilution to negatively affect growth performance of cattle. It is assumed that the dilution of energy with increasing roughage drives an increase in DMI, as cattle attempt to eat to meet their energetic demands (Benton et al., 2007). In the present study, DMI increased linearly as rNDF increased, but concentration of rNDF did not affect ADG. Because there were no low-S diets in the present study, an extrapolation of the effect of increasing rNDF on DMI of steers fed high-S diets compared with those consuming low-S diets cannot be made. However, a comparable study used 2 concentrations of S (0.28 and 0.56%) and 3 concentrations of grass hay (5, 10, and 15%, DM basis) to examine the effects on performance of feedlot cattle (Huber et al., 2012). Increasing roughage in the diet increased DMI, and increasing dietary S decreased

DMI (Huber et al., 2012). The authors reported no dietary S × roughage concentration interaction for any performance variables including DMI, ADG, and feed efficiency (Huber et al., 2012). In a feedlot growth-performance trial, Calderon-Cortes and Zinn (1996) fed a diet based on steam-flaked corn with dietary concentrations of roughage (8 and 16% sudangrass hay) similar to those of the present study (5.7 and 11.4% rNDF diets) and found that NEg in the diet, ADG, and feed efficiency worsened as roughage concentration in the diet increased. The discrepancy between studies evaluating the effect of roughage dilution of energy in feedlot diets on cattle performance may relate to the composition of the nonroughage portion of the diet. Because distillers grains are high in fiber there may be some positive associative effects of increasing roughage concentration on digestibility of distillers grains by supporting a more favorable environment for cellulolytic bacteria, potentially providing more energy to the animal. However, the implication of dietary S concentration on the relationship between coproduct digestibility and roughage content of the diet warrants further research.

We speculate the fiber fraction of DDGS was more thoroughly digested by cattle fed the higher roughage diets. Dried distillers grains with solubles are high in fiber; the DDGS used in the present study contained 25% NDF (DM basis). Morrow et al. (2013) investigated the effects of 7 or 14% hay (DM basis) in cracked corn- or DDGS-based finishing diets on ruminal fiber fermentation capabilities by measuring in situ DM disappearance of soybean hulls after 24 or 48 h incubation. Diets including DDGS had greater in situ DM digestion when compared to corn, and the inclusion of hay tended to increase soybean hull DM digestion in both diets, regardless of energy source (Morrow et al., 2013). We speculate that increased digestibility of fiber is due to favorable shifts in the rumen microbial ecology; however, it remains unclear what shifts are occurring due to increasing rNDF and the effect of these shifts on sulfate reducing bacteria and their metabolism. In the present study, including more rNDF in the diet may have created a more favorable rumen environment for cellulolytic bacteria and thus digestion of hay and also of the highly fibrous DDGS may have been more complete, helping to overcome the energy dilution of the diets when corn was replaced with hay.

In conclusion, cattle gains did not differ among treatments. The inclusion of up to 11.4% rNDF did not negatively affect cattle performance. With an increasing rNDF, DMI and ruminal pH increased linearly and H₂S concentration decreased linearly. However, there were limited benefits in increasing ruminal pH and decreasing H₂S concentration, in regards to cattle performance. The results of this study, in combination with our previous work, suggest that the inclusion of at least 7 to 8% rNDF in dry-rolled corn-based diets containing moderate amounts of S will aid

in modulating ruminal pH, thus decreasing ruminal H₂S gas concentration. Increasing roughage may be an effective feeding strategy to decrease the risk of S toxicity. However, modulation of ruminal pH is likely not the only management strategy that will aid in the prevention of S toxicity, as a variety of other factors such as dietary composition, feed bunk and intake management, and environment may all play roles in the onset of this problem. Future research is needed to identify additional factors influencing ruminal H₂S concentrations other than ruminal pH.

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