
Interaction of distillers grains sulfur concentration and dietary roughage on microbial fermentation

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TABLE OF CONTENTS

EXECUTIVE SUMMARY2

SUMMARY2

INTRODUCTION3

MATERIALS AND METHODS3

RESULTS AND DISCUSSION4

TABLES6

 TABLE 1. EFFECTS OF DIETARY ROUGHAGE AND SULFUR CONCENTRATIONS ON HYDROGEN SULFIDE, TOTAL GAS, AND PH PARAMETERS IN CONTINUOUS CULTURES OF RUMEN CONTENTS 6

 EXPERIMENT 2. EFFECTS OF DIETARY ROUGHAGE AND SULFUR CONCENTRATIONS ON NITROGEN PARAMETERS IN CONTINUOUS CULTURES OF RUMEN CONTENTS..... 7

LITERATURE CITED.....8

EXECUTIVE SUMMARY

High fat and high sulfur (S) concentrations in full-fat distillers grains plus solubles limit inclusion rates in feedlot cattle diets. High dietary S concentrations reduce intake and have a direct impact on the respiratory tract health. Subsequently, health and performance of cattle are affected by high S concentrations in distillers grains diets. Hydrogen sulfide is recognized to be the culprit; it results from reducing dietary sulfate in the rumen. Sulfate reduction to hydrogen sulfide is dependent on rumen pH. At lower rumen pH, sulfate is readily transformed to hydrogen sulfide. Several strategies have been used to prevent impact of high S in distillers grains on cattle health and performance. Use of roughage to encourage greater ruminal pH thereby reducing transformation of sulfate to hydrogen sulfide presents another opportunity to abate negative impact of hydrogen sulfide on cattle health and performance. This study represents the companion to a study where we investigated response to high sulfur concentrations in vivo (AIC 124). Due to limited research available evaluating the effectiveness of adding roughage to maintain a greater rumen pH and reduce transformation of sulfate to hydrogen sulfide, there is an urgent need to determine the rumen environment. Benefits of greater roughage inclusion would then justify its application as increased roughage diets are known to reduce feed efficiency and increase cost of gain.

We proposed this study to understand the mechanisms by which a greater supply of roughage in the rumen would lead to a reduction in the negative impact of sulfur on microbial fermentation. The hypothesis was that, compared with feeding DG with a high S concentration, feeding DG with a low S concentration would result in improved microbial fermentation and reduced H₂S concentration, and therefore allow for reduced dietary roughage concentrations and greater use of corn and DG.

Observations of rumen fluid pH in this study with a continuous fermentation system were indicative of normal pH and VFA response to increased energy supply to the rumen. Delivering a high-energy diet led to lower rumen fluid pH and greater total VFA and flow of ammonia N. Inclusion of greater concentrations of roughage in diets delivered to fermenters also behaved as expected; greater ruminal pH and lower concentrations of VFA. Interestingly, however, greater inclusion of roughage in diets delivered to the fermenters led to increased hydrogen sulfide concentration. Although we expected hydrogen sulfide concentration to decrease based on the hypothesis that greater rumen pH resulting from greater roughage inclusion would reduce transformation of sulfate to hydrogen sulfide; improved microbial activity resulting from greater pH could have supported greater gas production and conversion of sulfate to hydrogen sulfide. Therefore, we reject our hypothesis that greater roughage concentration should decrease transformation of sulfate to hydrogen sulfide through its effect of increasing rumen pH. Instead, greater rumen pH resulting from increased roughage concentration may act to enhance microbial activity thereby increasing gas and hydrogen sulfide production.

Results from this study aid in explaining why we did not see improved cattle performance when increasing dietary roughage in diets containing 0.53% S in a companion feedlot performance study (AIC 124). Together, results from these studies are taken to demonstrate that increasing roughage concentration in high-energy, high-S distillers grains diets do not aid in preventing a build-up of hydrogen sulfide in the rumen. Quite the contrary, increasing the concentration of roughage in distillers grains-based diets containing high concentrations of S has the potential of increasing negative effects of S.

Concurrently, current survey data from another project funded by Minnesota Corn Growers Association indicated that the ethanol industry has made tremendous progress in managing grain fermentation so that concentrations of S in resulting co-products are lower.

SUMMARY

A continuous culture fermenter experiment was conducted to determine the effect of dietary roughage and sulfur concentration on ruminal fermentation and hydrogen sulfide production when fed as a part of a feedlot-finishing diet. The experiment consisted of eight treatments: Treatment 1 (CON) was a control treatment consisting of (dry matter

basis) 86% corn, 9% roughage, and 5% supplement; treatments 2-4 consisted of a diet containing 52% corn, 40% DG, 3% roughage, and 5% supplement, with dietary S levels of 0.3% (LRLS); 0.4% (LRMS); and 0.5% (LRHS); treatments 5-7 consisted of a diet containing 46% corn, 40% DG, 9% roughage, and 5% supplement, with dietary S levels of 0.3% (MRLS); 0.4% (MRMS); and 0.5% (MRHS); and treatment 8 consisted of a diet with 28% corn, 40% DG, 27% roughage, and 5% supplement with a dietary S level of 0.5% (HRHS). Average pH of ruminal contents was lower (5.09; $P < 0.05$) for CON than for all other treatments, and was higher (5.33; $P < 0.05$) for HRHS than for all other treatments. The pH results agreed with VFA production results, which showed the highest VFA production with the CON treatment (226 mM; $P < 0.05$). Total H₂S production was lowest for the CON treatment (0.13 μg; $P < 0.05$), which was expected due to the lower dietary sulfur concentration in the CON treatment. Increasing dietary roughage concentration did not decrease H₂S production, and in fact H₂S production numerically increased as roughage concentration increased. This suggests that the increased roughage concentration in the MR treatments compared with the LR treatments may have provided for improved ruminal fermentation, and the improved fermentation offset any potential reductions in H₂S production.

INTRODUCTION

The increase in ethanol production has provided Minnesota feedlot operators a great opportunity to utilize ethanol co-products such as distillers grains (DG) in feedlot rations. Despite the known advantages of including high levels of DG in feedlot rations, there are concerns among feedlot producers about the potentially negative aspects of DG feeding that may limit its inclusion in feedlot diets (Crawford, 2007). The primary concern with feeding of high levels of DG is the high sulfur (S) content associated with DG. Concentration of S in DG ranges widely, from approximately 0.4% of the dry matter content of feed to over 1.0%. An excess of S in feedlot diets can lead to hydrogen sulfide (H₂S) toxicity, which may result in decreased animal performance, increased morbidity, and in acute cases, death (Kung et al., 1998; Gould et al., 2002).

The conversion of dietary S to H₂S in the rumen of cattle appears to be related to ruminal pH and dietary roughage level (NRC, 2005). An increase in dietary roughage concentration and reduction of starch in feedlot diets leads to an increase in ruminal pH (Crawford et al., 2008). This will decrease the conversion of dietary S to H₂S, but will also result in a reduction of volatile fatty acid production which can reduce cattle weight gains and feed efficiency. Because DG inclusion in feedlot diets is usually in place of corn grain, it is logical to assume that DG inclusion would lead to an increase in ruminal pH due to a reduction in dietary starch. The reduction in dietary starch, in turn, would logically allow for a reduction in dietary roughage concentration (Benton et al., 2007). However, because the conversion of S to H₂S is related to ruminal pH, many feedlot operators and nutritionists are hesitant to increase DG inclusion and reduce roughage concentrations in diets. Quantification of the interaction between dietary S concentration and dietary roughage could allow feedlot operators and nutritionists to decrease roughage concentrations in feedlot diets and increase the utilization of DG.

The objective of these experiments was to determine the interaction between dietary S concentration and dietary roughage on microbial fermentation. The hypothesis was that, compared with feeding DG with a high S concentration, feeding DG with a low S concentration would result in improved microbial fermentation and reduced H₂S concentration, and therefore allow for reduced dietary roughage concentrations and greater use of corn and DG.

MATERIALS AND METHODS

Eight dual flow, continuous culture fermenters were utilized to determine pH of ruminal contents, microbial fermentation, and H₂S production from cultures of ruminal microbes fed one of eight treatments. Fermenters were provided one of eight dietary treatments: Treatment 1 (CON) was a control treatment consisting of (dry matter basis) 86% corn, 9% roughage, and 5% supplement; treatments 2-4 consisted of a diet containing 52% corn, 40% DG, 3% roughage, and 5% supplement, with dietary S levels of 0.3% (LRLS); 0.4% (LRMS); and 0.5% (LRHS); treatments 5-7 consisted of a diet containing 46% corn, 40% DG, 9% roughage, and 5% supplement, with dietary S levels of 0.3% (MRLS); 0.4% (MRMS); and 0.5% (MRHS); and treatment 8 consisted of a diet with 28% corn, 40% DG, 27% roughage, and 5% supplement with a dietary S level of 0.5% (HRHS). Ruminal fluid was collected from a cannulated steer

consuming a high-concentrate diet and was allocated to each of the eight 1-L fermenters. Four 10-day periods were utilized resulting in a total of 32 observations and four replications per treatment. The first seven days of each period allowed for adaptation of microbes, and data collection occurred the final three days of each collection period. Data collected included volatile fatty acid concentration, microbial pH, H₂S production, nutrient digestion, and microbial protein production.

RESULTS AND DISCUSSION

Total H₂S production and concentration and pH results are shown in Table 1. Ruminal content pH in continuous culture was lower for the CON treatment (5.09) than all other treatments ($P < 0.05$). This could be expected due to the higher starch concentration of this treatment. The HRHS treatment, which contained 27% roughage, had a pH higher than all other treatments (5.33; $P < 0.05$). In general, the moderate (9%)-roughage treatments had a higher pH (average of 5.21 for MRLS, MRMS, and MRHS) than the low (3%)-roughage treatments (average of 5.17 for LRLS, LRMS, and LRHS treatments) which would be expected due to the higher roughage content of the 9% roughage diets.

Total H₂S production was numerically lower for the CON treatment (0.13 ug) compared with all other treatments (range of 0.82 ug to 3.85 ug); however, due to large variation among the observations this difference was only significant ($P < 0.05$) when comparing the CON treatment to the MRMS, MRHS, and HRHS treatments. Somewhat surprisingly, the HRHS treatment had numerically the highest H₂S production (3.85 ug), which was significantly ($P < 0.05$) higher than all treatments other than the MRMS and MRHS treatments. Our hypothesis was that an increase in dietary roughage would decrease the conversion of dietary sulfur to H₂S, which would therefore mean that the HRHS treatment would have a lower H₂S production than the MRHS and LRHS treatments. The lack of decrease in H₂S production with the HRHS treatment could indicate that the higher pH observed with the HRHS treatment could have promoted increased microbial fermentation, and therefore increased H₂S production compared with lower-roughage treatments.

We also hypothesized that increased dietary roughage concentration within dietary sulfur concentration would decrease H₂S production. At each dietary sulfur concentration, an increase in dietary roughage led to a numerical increase in H₂S production. This is the opposite response as we expected. This again could be due to the higher roughage concentration creating a more favorable environment for ruminal fermentation (ie. increased pH), and the increased fermentation resulted in increased H₂S production.

Table 2 shows the effects of dietary roughage and sulfur concentrations on nitrogen parameters in continuous culture of ruminal contents. Ammonia-N concentrations were higher in the CON treatment (4.75 mg/dL) than all other treatments ($P < 0.05$). The CON treatment contained a protein supplement that consisted of 65% CP, 50% of which was ruminally-degradable protein, and much of this protein was non-protein nitrogen (NPN). The DG-containing treatments received the majority of their CP from DG, and contained very little NPN. Therefore, it is expected that the CON treatment had a greater ammonia-N concentration than all other treatments. No other significant statistical differences were observed for nitrogen flow, suggesting that dietary concentrations of roughage and sulfur had little effect on microbial nitrogen synthesis.

Total VFA production is also shown in Table 2. Total VFA production was highest ($P < 0.05$) with the CON treatment, which was expected due to the higher starch concentration in the CON treatment. Distillers grains contain very little starch and 10-12% fat. Fat has 2.25 times the amount of energy per unit as carbohydrate. However, much of the added benefit of fat compared with starch is realized post-ruminally, as the fat will be absorbed in the small intestine rather than digested in the rumen. Starch, on the other hand, is rapidly and nearly completely digested in the rumen, providing substrate for ruminal microbes for VFA production. Further evidence of the effect of starch on ruminal VFA production is shown by the VFA production response to the HRHS treatment, which was lower than all treatments other than the MRLS and MRHS treatments. The HRHS treatment contained very little starch, and this corresponded with low VFA production.

In summary, our hypothesis that increased dietary roughage concentration would allow for greater dietary concentrations of sulfur without increasing H₂S production was not proven entirely correct. However, it appears that

any added protection to the rumen by additional roughage may have been offset by the improved pH with added roughage providing a more favorable environment for ruminal fermentation.

Table 1. Effects of dietary roughage and sulfur concentrations on hydrogen sulfide, total gas, and pH parameters in continuous cultures of rumen contents¹.

Item	CON	LRLS	LRMS	LRHS	MRLS	MRMS	MRHS	HRHS	SEM ²	P-value
Total H ₂ S production	0.13 ^a	0.82 ^{ac}	1.26 ^{ac}	1.48 ^{ac}	1.09 ^{ac}	2.38 ^{bc}	2.63 ^{bc}	3.85 ^b	1.03	0.04
H ₂ S concentration	0.04 ^b	0.27 ^{bc}	0.42 ^{bc}	0.49 ^{bc}	0.36 ^{bc}	0.79 ^a	0.88 ^{ac}	1.28 ^a	0.34	0.04
µg H ₂ S/g diet DM	0.00 ^a	0.01 ^{ac}	0.02 ^{ac}	0.02 ^{ac}	0.01 ^{ac}	0.03 ^{bc}	0.04 ^{bc}	0.05 ^b	0.01	< 0.05
pH	5.09 ^a	5.12 ^d	5.21 ^e	5.17 ^c	5.16 ^c	5.21 ^e	5.23 ^e	5.33 ^b	0.01	<0.01

¹ CON = Control, no DG, 9% roughage, 0.18% S; LRLS = 3% roughage, 0.30% S; LRMS = 3% roughage, 0.40% S; LRHS = 3% roughage, 0.50% S; MRLS = 9% roughage, 0.30% S; MRMS = 9% roughage, 0.40% S; MRHS = 9% roughage, 0.50% S; HRHS = 27% roughage, 0.50% S. All treatments other than CON contained 40% dried DG.

² Standard error.

Table 2. Effects of dietary roughage and sulfur concentrations on nitrogen parameters in continuous cultures of rumen contents¹.

Item	CON	LRLS	LRMS	LRHS	MRLS	MRMS	MRHS	HRHS	SEM ²	P-value
Total VFA (mM)	225.99 ^a	181.74 ^b	168.79 ^{bd}	167.14 ^{bd}	170.56 ^{bd}	182.22 ^b	145.43 ^{cd}	159.72 ^{bd}	16.26	<0.01
NH ₃ -N (mg/dL)	4.75 ^a	1.10 ^{bc}	1.06 ^{bc}	2.90 ^{ac}	1.20 ^{bc}	1.68 ^{bc}	2.62 ^{bc}	2.41 ^{bc}	0.91	<0.01
N flow (g/d)										
NH ₃ -N	0.09 ^a	0.02 ^b	0.02 ^b	0.06 ^{ac}	0.02 ^{bc}	0.03 ^{bc}	0.05 ^{bc}	0.05 ^{bc}	0.02	<0.01
Non NH ₃ -N	2.22 ^a	2.43 ^b	2.41 ^b	2.36 ^b	2.37 ^b	2.25 ^b	2.30 ^b	2.23 ^b	0.33	0.01
N										
Microbial-N	1.40 ^{ac}	1.31 ^{ac}	1.69 ^{ac}	1.87 ^a	1.12 ^{bc}	1.65 ^{ac}	1.58 ^{ac}	1.35 ^{ac}	0.28	0.21
N										
Dietary-N	0.82 ^a	1.12 ^{bcd}	0.72 ^{ac}	0.52 ^{ad}	1.25 ^{bc}	0.62 ^{ac}	0.72 ^{ac}	0.87 ^{ac}	0.33	0.19
CP degradation (%)	60.71 ^a	47.64 ^{bc}	66.80 ^{ac}	75.69 ^{acd}	40.91 ^{bd}	70.36 ^{abc}	65.90 ^{abc}	58.23 ^{acd}	15.49	0.19

¹ CON = Control, no DG, 9% roughage, 0.18% S; LRLS = 3% roughage, 0.30% S; LRMS = 3% roughage, 0.40% S; LRHS = 3% roughage, 0.50% S; MRLS = 9% roughage, 0.30% S; MRMS = 9% roughage, 0.40% S; MRHS = 9% roughage, 0.50% S; HRHS = 27% roughage, 0.50% S. All treatments other than CON contained 40% dried DG.

² Standard error.

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