Dietary Sulfur Concentration Has No Effect On *In Vitro* Fermentative Activity of Ruminal Mixed Microorganisms

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Introduction

We previously reported that elevated concentrations of dietary sulfur (0.65% sulfur, dry basis) in finishing diets containing dried distillers grains with solubles decreased dry matter intake and average daily gains of feedlot cattle. Furthermore, high dietary sulfur concentrations yielded lower ruminal concentrations of volatile fatty acids, but were associated with increased ruminal ammonia concentrations and improved total tract digestibility of the diet. The objective of this study was to investigate, in culture tubes, effects of added sulfur on *in vitro* dry matter disappearance, volatile fatty acid profiles, and ammonia concentrations from substrates containing different sulfur concentrations when fermented by mixed ruminal microorganisms from a steer fed a diet based on corn and alfalfa.

Experimental Procedures

A study was conducted in culture tubes to evaluate effects of adding sulfur from sodium sulfate at 0, 0.1, 0.2, 0.3, 0.4, 0.5, or 0.6% of substrate (dry basis) on fermentative activity of mixed ruminal microorganisms from a steer fed a diet based on corn and alfalfa. Substrates consisted of a 94:4.5:1.5 mixture of ground corn, soybean meal, and urea, or a 69.4:30.6 mixture of ground corn and distillers grains. Basal sulfur concentrations were 0.18% (dry basis) for the corn-soybean meal-urea mixture and 0.28% for the corn-distillers grains mixture. Both substrates contained 14.4% crude protein (dry basis). Sulfur concentrations were increased experimentally to allow evaluation of potential threshold concentrations at which dietary sulfur might depress microbial activity. Varying concentrations of sulfur were added to substrates prior to incubation in culture tubes containing a 2:1 mixture of artificial saliva and clarified ruminal fluid from a single donor.

The study was repeated daily for 3 days. Each day, three tubes per substrate and sulfur concentration were incubated for 24 hours at 102°F. The steer used as the source of ruminal fluid was fed a diet containing (dry basis) 49% ground corn, 40% alfalfa hay, 5% corn steep liquor, and 6% of a supplement that provided 300 mg/day of Rumensin and 90 mg/day of Tylan (Elanco Animal Health, Greenfield, IN). The diet contained 13.3% crude protein and 0.19% sulfur (dry basis). After 24 hours of fermentation, tubes were chilled in an ice bath and centrifuged, and the supernatant was used for analysis of volatile fatty acid profiles and ammonia concentrations. Pellets of residue were dried and used to determine *in vitro* dry matter disappearance.

Results and Discussion

No interactions occurred ($P>0.05$) between sulfur level and substrate type with respect to concentrations of ammonia (Figure 1), total volatile fatty acids (Figure 2), acetate, propionate, butyrate, isovalerate, isobutyrate, valerate, and lactate; acetate:propionate

ratio; or *in vitro* dry matter disappearance (Figure 3). These parameters also were unaffected by sulfur concentration ($P>0.05$). Microorganisms fed ground corn with distillers grains produced greater concentrations of valerate compared to microbes fed corn and soybean meal, but produced lower concentrations of ammonia (Figure 1), total volatile fatty acids, propionate, and butyrate and had lower *in vitro* dry matter disappearance than microorganisms fed a mixture of ground corn, soybean, and urea ($P<0.05$; Figure 3).

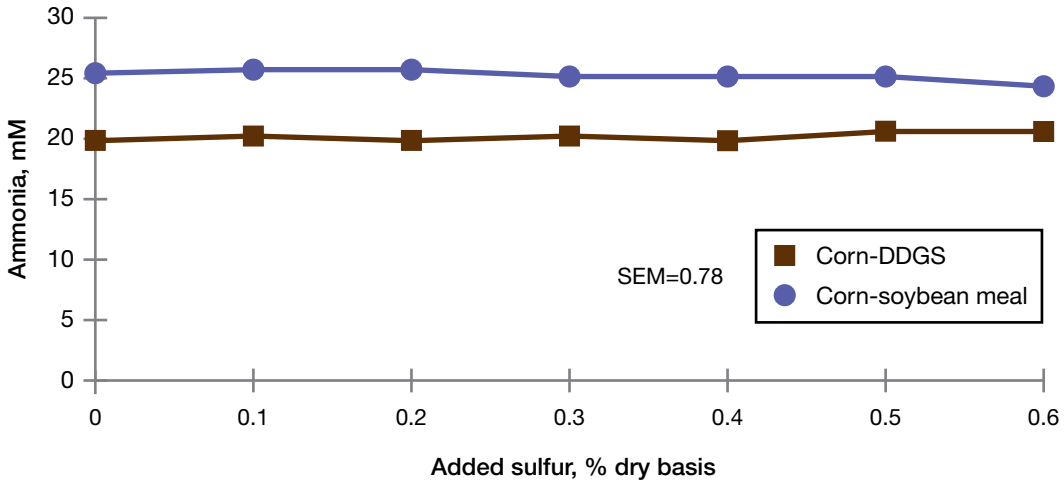
Lack of effect of dietary sulfur on ruminal fermentation parameters and *in vitro* dry matter disappearance has been previously reported with sulfur concentrations of 0.2, 0.4, and 0.8% of a steam-flaked corn-based substrate (dry basis). In our study, as well as in the previous work, dietary sulfur had no effect on ruminal fermentation parameters. Ruminal fluid in our study was from a steer fed a high-roughage diet, whereas previous work used fluid from animals fed a high-concentrate diet. Collectively, these studies suggest that microbial populations are unaffected by concentration of dietary sulfur.

The present study was designed to allow direct comparison between two substrates. Our intent was to identify threshold concentrations at which dietary sulfur depresses microbial activity under different situations, but we observed no effects of sulfur with either substrate. These results suggest that previously observed changes in cattle performance and *in vivo* diet digestibility associated with high sulfur intake are likely attributable to host factors such as feed intake. Elevated dietary sulfur depresses feed intake, which leads to poor growth performance.

Differences between substrate types may be attributable to differences in the content of fiber (neutral detergent fiber) and ruminally degradable protein. The ground corn-soybean meal-urea mixture provided more degradable protein but less fiber compared to the ground corn-distillers grains mixture. Hence, improved diet digestibility was observed in cultures incubated with ground corn-soybean meal-urea mixture compared to cultures incubated with the ground corn-distillers grains mixture.

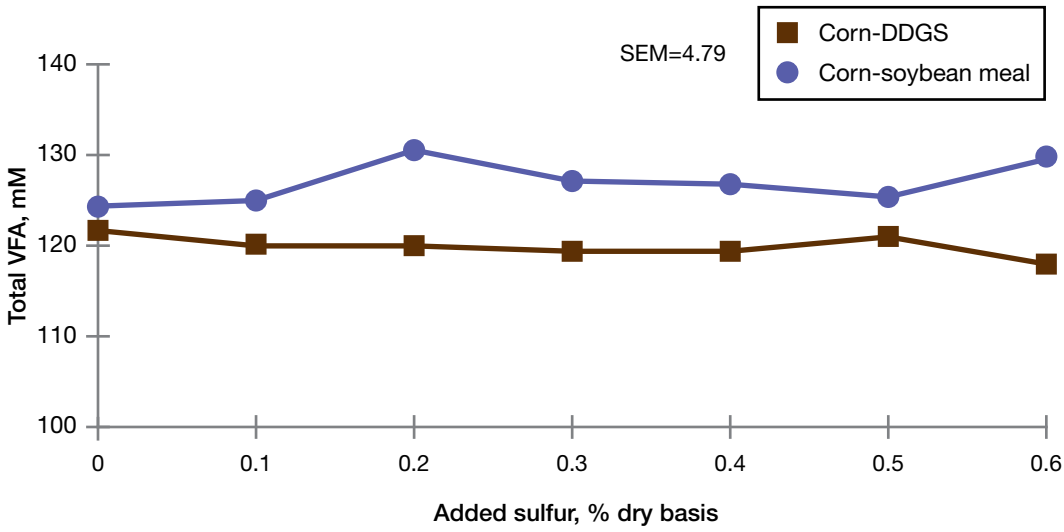
Implications

Substrates yielded marked differences in fermentative end products, but elevated sulfur did not alter fermentation of these substrates by mixed ruminal microorganisms in culture tubes. Previously observed deleterious effects of elevated dietary sulfur on cattle growth performance are unlikely to be the result of impact on gut microbes.



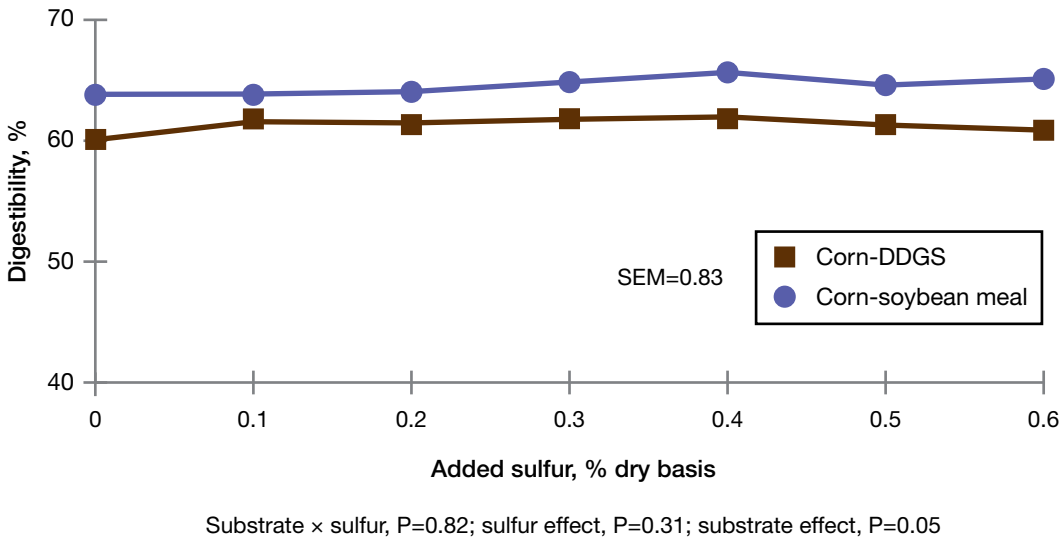
Substrate × sulfur, P=0.32; sulfur effect, P=0.95; substrate effect, P<0.01

Figure 1. *In vitro* ammonia concentration of substrates with or without distillers grains with solubles (DDGS) with increasing concentrations of sulfur.



Substrate × sulfur, P=0.11; sulfur effect, P=0.80; substrate effect, P=0.09

Figure 2. *In vitro* total volatile fatty acid (VFA) concentration of substrates with or without distillers grains with solubles (DDGS) with increasing concentrations of sulfur.



Substrate × sulfur, P=0.82; sulfur effect, P=0.31; substrate effect, P=0.05

Figure 3. *In vitro* dry matter disappearance of substrates with or without distillers grains with solubles (DDGS) with increasing concentrations of sulfur.