UTILIZATION OF DISTILLERS GRAINS IN FEEDLOT CATTLE DIETS

by

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B.S., National University of Rwanda, 2002
M.S., Kansas State University, 2008

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

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Abstract

Four studies evaluated effects of dry distillers grains with solubles (DDGS) containing high S concentrations on feedlot performance, ruminal fermentation, and diet digestibility by finishing cattle. Trial 1 used finishing steers fed diets based on steam-flaked corn (SFC) or dry-rolled corn (DRC), and containing 30% DDGS (DM) with 0.42% S (0.42S) or 0.65% S (0.65S). No interaction ($P \geq 0.15$) between dietary S and grain processing occurred, but feeding 0.65S decreased DMI ($P < 0.001$) and ADG ($P = 0.006$) by 8.9% and 12.9%, respectively, whereas G:F was unaffected by S concentration ($P = 0.25$). Steers fed 0.65S had 4.3% lighter HCW ($P = 0.006$), lower KPH ($P = 0.009$), and lower yield grades ($P = 0.04$) than steers fed 0.42S. Concentration of H_2S was inversely related ($P \leq 0.01$) to ADG (r = -0.58) and DMI (r = -0.67) in cattle fed SFC, and DMI (r = -0.40) in cattle fed DRC. Trial 2 used the same treatments as in the first stud, and investigated ruminal fermentation characteristics and diet digestibility by feedlot cattle. Feeding 0.65S increased ruminal pH ($P < 0.05$), but decreased total VFA concentrations ($P = 0.05$). Steers fed 0.65S had greater ruminal NH_3 concentrations ($P < 0.01$) than steers fed 0.42S. The magnitudes of these effects were greater in steers fed DRC than in steers fed SFC (interaction, $P < 0.01$). Feeding 0.65S yielded greater apparent total tract digestibilities of DM ($P = 0.04$) and ether extract ($P = 0.03$). The 3rd study evaluated effects of *in vitro* S titration (0, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6% of DM) in substrates based on ground corn and DDGS (GC-DDGS) or ground corn with urea and soybean meal (GC-SBM). Concentrations of NH_3, total VFA, IVDMD, *in vitro* gas production, and gas composition were unaffected by S ($P > 0.05$) or by the S × substrate interaction ($P > 0.05$). Study 4 evaluated cattle feedlot performance when exposed to DDGS containing high S levels, either continuously or intermittently. Treatments were chronic high S (CHS; 0.60% DM), chronic intermediate S (CIS; 0.50% DM), and sporadic intermediate S (SIS; oscillating from 0.40 or 0.60% S DM basis). Steers fed CHS had 11.2 and 6.1% less ($P < 0.05$) DMI than steers fed CIS and SIS, respectively, but there were no treatment effects on ADG, G:F, or carcass characteristics ($P > 0.10$). These studies suggest that changes in cattle performance and digestibility associated with high S are primarily attributable to decreased DMI, but infrequent exposure to high levels is no more harmful than continuous exposure.

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Dedication

I dedicate this dissertation to my Lord and Savior Jesus Christ Who has led my every step of the way, and will never leave nor forsake me.

Psalm 126:3

The LORD has done great things for us, and we are filled with joy.

Psalm 103:1-2

Bless the LORD, O my soul;
And all that is within me, bless His holy name!
Bless the LORD, O my soul,
And forget not all His benefits.

Chapter 1 - Introduction

Distillers grains with solubles (DGS) is a co-product of dry milling of cereal grain to make ethanol (Klopfenstein et al., 2008). During the dry milling process, grain is ground, soaked, and cooked before enzymes and yeast are added to the mixture to ferment sugars into ethanol. Ethanol is subsequently removed via distillation, and the resulting by-product called stillage is centrifuged to separate distillers grains (solid fraction of stillage) from distillers solubles (liquid portion of the stillage). After evaporation of water from solubles, distillers grains and condensed solubles are blended together to make DGS in the wet (WDGS) or dry (DDGS) forms (Shurson et al., 2005).

Rapid expansion of the US ethanol industry has prompted a number of cattle producers to routinely utilize DGS as an inexpensive source of dietary protein and energy for cattle (Vasconcelos and Galyean, 2007). Although DGS is an economically attractive replacement for cereal grain in finishing diets, the feedlot industry has been concerned with inconsistency of its nutrient composition (Buckner et al., 2011).

Sulfuric acid is added to fermenters to control pH for optimum ethanol yield. Consequently, a wide range of sulfur (S) concentration in DGS has been reported from 0.33 to 0.74% DM among dried DGS (Spiehs et al., 2002; Holt and Pritchard, 2004), and 0.36 - 0.60% for wet DGS (Holt and Pritchard, 2004; Buckner et al., 2011). According to Holt et al. (2004), condensed distillers solubles may contain from 0.8 to 1% S on a DM basis. Dietary S content of feedlot diets increased linearly with increasing inclusion of DGS from 0 to 40% of diet DM (Corrigan et al., 2009). In a study by Buckner et al. (2007), 9 out of 50 animals fed dry-rolled corn finishing diets containing 50% DGS (0.6% S) were diagnosed with polioencephalomalacia (PEM), and some of them died. Polioencephalomalacia is a neurological disorder characterized by necrosis of the cerebral cortex (Gould, 1998). Clinical symptoms of PEM include increased respiration rates, progressive blindness, depressed feed intake, head pressing, and the animal's breath has a smell of rotten eggs (Gould, 1998). Kung et al. (1998) suggested that elevated ruminal hydrogen sulfide (H_2S) concentration can cause PEM. Hydrogen sulfide is an end product of microbial sulfate reduction in the rumen (Bray and Till, 1975). Sulfur metabolism in the rumen and its effects on ruminal microbial activity and physiology of the animal host is further discussed in subsequent paragraphs.

Sulfur Requirements

Sulfur is an important mineral for optimal growth and overall physiology of cattle. It is required by ruminal microorganisms to synthesize S-containing amino acids such as methionine, cysteine, and cystine (NRC, 2000). Many other compounds, such as thiamine, biotin, taurine, cysteic acid, lipoic acid, coenzyme A, gluthathione, homocysteine, chondroitin sulfate, ergothionine, heparine, and estrogens can be synthesized from methionine *in vivo* (NRC, 1980). Sulfur deficiency compromises optimal microbial activity in the rumen and leads to anorexia, and causes excessive salivation, weight loss, and poor growth performance (NRC, 2000). Sources of S for livestock include drinking water (Loneragan et al., 2001), mineral salts, grains, forages, molasses, and ethanol co-products (NRC, 2000). Unlike monogastrics, requirements of S for ruminants are not well known, but NRC (2005) recommends a maximum tolerable level of 0.30% S (DM basis) for beef cattle fed high-concentrate diets and no more than 0.50% S for beef cattle fed high-roughage diets.

Sulfate Metabolism in the Gastrointestinal Tract

Ruminal microorganisms are able to synthesize most of their S-containing organic compounds by reducing inorganic sulfate to various forms of sulfide via assimilatory or dissimilatory pathways (Kandylis, 1984). Assimilatory sulfate reducing bacteria, such as *Bacteroides, Butyrivibrio, and Lanchnospira* (Emery et al., 1957), reduce sulfate to relatively small amounts of sulfide needed to satisfy their metabolic needs (Cummings et al., 1995). According to Block et al. (1951), this process is inhibited by concentration of S-containing compounds such as cysteine. Unlike assimilatory bacteria, dissimilatory sulfate reducing bacteria, such as *Desulfovibrio and Desulfotomaculum* spp (Stewart at al. 1988), derive energy for growth and metabolism from sulfate reduction. They are Gram negative, strictly anaerobic and produce H_2S in amounts that exceed their S requirements (Cummings et al., 1995).

Both assimilatory and dissimilatory sulfate reducing bacteria are present in the rumen, but dissimulatory bacteria outnumber assimilatory bacteria (Huisingh et al., 1974). Hence, the dissimilatory sulfate reduction pathway is believed to be the predominant means of sulfate reduction in the rumen, (Kandylis, 1984). Contrary to assimilatory sulfate reducing bacteria, activity of dissimilatory sulfate reducing bacteria is unaffected by S-containing compounds, but it is suppressed by excessive sulfide concentration (Peck and Lissolo, 1988). Ruminal pH of 6.5

was reported to be optimal for sulfate reduction (Kandylis, 1984). Previous research has reported other non-sulfate reducing ruminal bacteria that are able to produce H_2S from S-containing compounds because they have cysteine desulfhydrase (Cummings et al., 1995). These include *E. coli* (Metaxas and Delwiche, 1955), *Megasphaera sp, Selenomonas, Anaerovibrio,* and *Clostridium spp* (Cummings et al., 1995).

Ruminal sulfide is either incorporated into microbial proteins, absorbed by ruminal mucosa, or eructated. Previous research reported a wide range (45 to 67%) of ruminal sulfide incorporation into microbial proteins (Gawthorne and Nader, 1976; Kennedy and Milligan, 1978). According to Kennedy and Milligan (1978), 90% of protozoal S is of bacterial origin. Thus, ruminal sulfide is believed to be the major substrate from which ruminal microorganisms synthesize their S-containing amino acids *de novo* (Nader and Walker, 1970).

Sulfide that is not utilized by ruminal bacteria is rapidly absorbed across the ruminal wall with a half life of 10 to 22 min (Kandylis, 1984). Bray and Till (1975) reported a faster absorption rate of H_2S compared with that of hydrosulfide (HS^-). Ruminal sulfide concentration and pH dictate absorption rate (Beauchamp et al., 1984). According to Bray and Till (1975), 97.2% of ruminal sulfide pool was H_2S vs. only 2.8% as HS^- at pH 5.2, which approximates ruminal pH of cattle fed grain-based diets (May et al., 2009). At pH 6.8, which is more characteristic of the ruminal pH of cattle fed high-roughage diets, ruminal sulfide was evenly distributed between H_2S (46.8%) and HS^- (50.4%; Bray and Till, 1975). The pKa for HS^- is 7.04 (Beauchamp et al., 1984); hence, there is more H_2S in the ruminal gas cap when pH is below 7.0. Absorbed H_2S is oxidized to sulfate in blood or liver (Bray and Till, 1975). Sulfates contained in extracellular fluid can be recycled to the rumen via saliva (Kandylis, 1984).

A portion of ruminal H_2S can be eructated and substantial amounts of eructated H_2S can be respired into the lungs (Dougherty and Cook, 1962). Inhaled H_2S has been associated with respiratory problems and PEM (Gould, 1998). In a study by Loneragan et al. (1997), pulmonary arterial pressure increased with elevated H_2S production in the rumen. Research by Niles et al. (2000) reported that 150 stocker calves grazing pasture were diagnosed with PEM as result of *ad libitum* intake of a supplement consisting of a 50:50 blend of wet corn gluten feed (containing 2,300 mg/kg S) and soybean hulls. Out of 150 animals with PEM, 25 became chronically blind and 11 died (Niles et al., 2000).

Plasma proteins, mucous and sloughed mucosal cells have been reported to provide S post-ruminally, which is either absorbed across the intestinal wall or metabolized to H_2S by microbes in the ileum (Kandylis, 1984).

Effects of Sulfur on Metabolism of other Nutrients

Sulfur metabolism has been reported to interfere with metabolism of other minerals, nitrogen, and lactate.

Sulfur and Minerals

Elemental S possesses anionic properties and it is included in calculations of dietary cation-anion difference (DCAD). Dietary cation-anion difference is calculated as the balance between millequivalents of cations (K^+ and Na^+) and anions (Cl^- and S^{-2}), and can be used to assess metabolic effects of dietary minerals on cattle performance (Tucker et al., 1991). Low DCAD resulting from increasing dietary S decreased DMI in dairy cows (Tucker et al., 1991), but effects of dietary S as a component of DCAD on performance of beef cattle were less conclusive. In studies by Ross et al. (1994a,b) DMI by growing and finishing steers were improved by 8.5 and 13.8%, respectively, when dietary DCAD was increased from 0 to 15 mEq/ 100 g DM, but DCAD increased beyond 15 mEq/100 g DM yielded no DMI improvement. Spears et al. (2011) found no performance improvement of finishing cattle by increasing DCAD from -16.6 to - 2.2 mEq/100 g DM. Qi et al. (1993) reported no effect of DCAD on performance of growing goats.

Sulfur also has been reported to sequester dietary Cu through formation of insoluble CuS, thus decreasing Cu bioavailability in cattle (Suttle, 1974; Spears, 2003). Spears et al. (2011) added 10 mg Cu/kg DM to corn-based finishing diets containing various S concentrations. These researchers reported that steers fed 0.46% S had lower concentrations of plasma and liver Cu compared with steers fed 0.31% S, but had no Cu deficiency (NRC, 2005). Conversely, research by Cammack et al. (2010) reported that liver and plasma Cu concentrations dropped to near Cu deficiency when dietary S was increased from 0.29 to 0.73% S in a diet without added Cu.

Literature also reported decreased bioavailability of dietary Mo, S, and Cu due to formation of insoluble $CuMoSO_4$ (Whanger, 1972; Spears, 2003). Moreover, an interaction between Mo and S has been reported by a number of researchers. In a study by Bryden and Bray (1972), supplementation of 40 mg/kg Mo to sheep increased ruminal sulfide concentration, but when Mo

supplementation was increased from 240 to 1440 mg/kg, ruminal sulfide was sharply decreased, though VFA production was not affected. Similarly, 50 mg/kg of added Mo increased sulfide concentration both in cattle (Kandylis, 1984) and sheep (Gawthorne and Nader, 1976). Conversely, research by Huisingh et al. (1975) indicated that 50 mg/kg Mo inhibited sulfide production from sulfate reduction, but increased sulfide production from methionine. Thus, Huisingh et al. (1975) suggested that discrepancies between their findings and those reported by Kandylis et al. (1984) and Gawthorne and Nader (1976) could reflect degradation of S-containing amino acids in the rumen.

Elevated dietary S also decreases Se bioavailability which could increase incidences of white muscle disease in large animals (Spears, 2003). Results from early research were conflicting about effects of dietary S on prevention of white muscle disease by supplemental Se (Hintz and Hogue, 1964; Whanger et al, 1969). According to Kandylis (1984), microbial uptake of selenomethionine was inhibited more by methionine and cysteine than sulfite in the rumen. Recently, Spears et al. (2011) reported reduced activity of a Se metalloenzyme when supplemental dietary S was increased from 0 to 0.31% of DM in finishing diets based on ground corn. Increasing dietary S from 0.21 to 0.70% also was associated with a linear decrease of plasma Se concentrations in lactating dairy cows (Ivancic and Weiss, 2001).

Sulfur and Nitrogen

There is evidence for interactions between metabolism of N and S in the rumen (Kandylis, 1984). Early study by Thomas et al. (1951) demonstrated suppression of NPN utilization by sheep as a result of S deficiencies, whereas increasing dietary S improved utilization of urea and N retention by sheep (Goodrich and Tillman, 1966). Feeding dietary S within the recommended levels (NRC, 2005) improved growth performance of growing cattle (Bolsen et al., 1973; Pendlum et al., 1976; Rumsey, 1978). However, Thompson et al. (1972) reported no interaction between concentration of S (from elemental S) and N source on growth performance of calves, when they decreased N:S ratio from 15:1 to 5:1. Lack of interaction may be attributable to the inefficient use of elemental S by ruminal microorganisms due to its low bioavailabilty (Kahlon et al., 1975). Sulfur contributes to improving microbial protein yield, overall fermentation activity and diet digestibility (Hume and Bird. 1970) due to its involvement in microbial synthesis of cysteine and methionine (Block et al., 1951; Thomas et al., 1951).

Ratios between 13.5 and 15:1 N:S have been recommended to ensure adequate S supply for optimal microbial protein yield in cattle supplemented with dietary NPN (Bird, 1974). Additionally, about 3.8 mg/L of ruminal sulfide is required per 85 mg/L of ruminal ammonia for optimal ruminal microbial efficiency (Kang-Meznarich and Broderick, 1981; Kandylis, 1984).

Sulfur and Lactate

Increasing dietary S promotes conversion of lactic acid to acrylyl-CoA, a S-containing intermediate for VFA production (Russell, 2002), via the acrylate pathway (Whanger and Matrone, 1967). According to Widdle (1988), sulfate-reducing bacteria that partially oxidize lactate thrive on lactate in the presence of elevated sulfate concentrations.

Previous research reported conflicting results with respect to effects of dietary S on lactate metabolism. Whanger and Matrone (1966) reported accumulation of lactate in the rumen of sheep fed S deficient diets, whereas lactate was metabolized to VFA in the rumen of sheep whose dietary S requirements were satisfied. Conversely, research by Rumsey (1978) noted a reduction in ruminal lactate concentrations in steers fed 0.28 vs. 0.14% dietary S, probably because steers were deprived of feed for 96 h before experimental diets were fed. In a study by Zinn et al. (1997), increasing S from 0.15 to 0.25% DM had no effect on ruminal lactate concentration in feedlot steers. Similarly, Qi et al. (1993) reported no effect of dietary S concentration on ruminal lactate concentrations in growing goats fed diets containing 0.11, 0.20, 0.28, or 0.38% S of diet DM. Dar et al. (2008) suggested that the proportion of lactate concentration relative to sulfate concentration impacts ruminal VFA profiles. Sulfur promotes propionate production by converting lactate to acrylyl-CoA, a S-containing intermediate (Russell, 2002), through acrylate pathway (Whanger and Matrone, 1967).

Effects of High Sulfur Concentration on Cattle Performance and Health

Sulfur Effect on Cattle Performance

Efficiency of dietary S utilization is a function of amount provided, sulfide production rate, bacterial uptake of ruminal sulfide, and rate of sulfide disappearance from the rumen either by absorption or eructation and inhalation (Kandylis, 1984). Increasing dietary S from 0.18 to 0.19, 0.22, 0.29, or 0.40% (Loneragan et al., 2001), or from 0.15 to 0.20, or 0.25% S (Zinn et al., 1997), decreased DMI and ADG of feedlot cattle when supplemental S was provided in sulfate

form, but dietary S had no effect on these parameters when elemental S was provided at 0.12 or 0.37% S (Thompson et al., 1972) or between 0.11, 0.26, or 0.45% S (DM basis, Pendlum et al., 1976). Excessive H_2S produced from sulfate reduction in the rumen suppresses ruminal and intestinal motility, hence the decrease in DMI (Bird, 1972).

Literature suggests bioavailability of S from different sources plays a role in efficiency of S microbial utilization. According to Kahlon et al. (1975), L-methionine was 100% available for microbial protein synthesis, followed by $CaSO_4$ (94.1%), $(NH_4)_2SO_4$ (93.0%), DL-methionine (63.0%), sulfate (50%); elemental S (35.8%); and hydroxyl analog of methionine (28.8%).

Sulfur Effects on Cattle Health

Although sulfide toxicity often is associated with PEM incidences (Raisbeck, 1982; Gould et al., 1997; McAllister et al., 1997), it is not the only metabolic disorder associated with elevated S concentration. In research by Short and Edwards (1989), high sulfide inhibited activity of many enzymes such as dipeptidases, dopa-oxidase, carbonic anhydrase, peroxidases, dehydrogenases, and catalases. Elevated sulfide also decreased ATP production by suppressing cytochrome c-oxidase activity (Kung, 2000). Additionally, sulfides adversely affect respiration due to their paralyzing effects on the carotid body (Bulgin et al., 1996). Sulfides also decrease blood oxygenation via formation of sulfhemoglobin (Beauchamp et al., 1984). According to Loneragan et al. (2001), high concentration of sulfide in blood may induce cell damage and suppress growth and overall cattle performance.

Scientists have investigated means of mitigating ruminal H_2S accumulation. Increasing anthraquinone concentrations *in vitro* decreased H_2S production linearly by preventing sulfate reduction to adenosine-5'-phosphosulfate (Kung et al., 1998). Molybdenum also has been used to decrease H_2S concentration via formation of thiomolybdate (Loneragan et al., 1998).

Distillers Grains with Solubles as a Sulfur Source

Effects of High-Sulfur DGS on Cattle Performance and Health

Sarturi et al. (2010) evaluated effect of 0.33, 0.43, and 0.54 % S from WDGS or DDGS on performance of finishing steers. Increasing dietary S decreased DMI more so with WDGS than DDGS but WDGS improved G:F. In a study by Wilken et al. (2009), 6% of cattle fed diets containing 0.47 to 0.59% S DM from blends of WDGS and wet corn gluten feed died or showed

symptoms of PEM. Similarly, 15% of steers fed 50% DDGS (with 0.60% S) in a dry-rolled corn (DRC)-based diet showed symptoms related to S toxicity (Buckner et al., 2007). A meta analysis by Vanness et al. (2009) revealed an increased likelihood of PEM incidence when diets containing ethanol by-products exceeded 0.56% S (DM basis). Using roughage titration down from 7.5 to 3.5% of diet DM, Vanness et al. (2009) also evaluated effects of ruminal pH on ruminal H_2S concentrations when cattle were fed diets containing 50% WDGS with 0.44, 0.46, 0.48, or 0.50% S through the transition period. Ruminal H_2S concentrations increased linearly with decreasing roughage levels.

Effects of High-Sulfur DGS on Microbial Fermentation

Data pertaining to the effects of high-S DGS on ruminal microbial activity *in vivo* are still lacking, but recently a number of studies have been conducted *in vitro*. DeClerk (2009) evaluated effects of feeding 15 or 30% (DM) corn or sorghum WDGS in steam-flaked corn (SFC)-based substrates on *in vitro* H_2S production. Sulfur contents for substrates were 0.20 or 0.26; and 0.19 or 0.31% S for corn WDGS and sorghum WDGS containing 15 or 30% WDGS (DM), respectively. Substrates containing WDGS produced more H_2S with increasing WDGS content from 15 to 30% of DM.

Leibovich et al. (2009) evaluated effects of 0 or 15% sorghum DGS on *in vitro* gas production and H_2S concentrations when combined with SFC or DRC. Dietary S concentrations were 0.21 or 0.23% DM of DRC-based diets, and 0.20 or 0.24% DM of SFC based diets, for 0 and 15% sorghum DGS, respectively. No substrate type × DGS level interaction was reported, but including sorghum DGS decreased gas production, but *in vitro* H_2S production was not affected by sorghum DGS level (Leibovich et al., 2009). May et al. (2011) evaluated effects of 15 or 30% (DM) WDGS and 7.5, 10, or 12.5% (DM) alfalfa in SFC-based substrates on ruminal microbial fermentation *in vitro*, but observed no interaction between WDGS and roughage level. Substrates containing WDGS yielded less total gas production, but more H_2S production than substrates without WDGS. Total gas production, rate of gas production, and IVDMD decreased with increasing WDGS in substrates, but WDGS had no effects on VFA profiles.

In an *in vitro* study by Quinn et al. (2009), H_2S concentration increased when S concentration was increased from 0.17 to 0.42% of SFC-based substrate DM, but IVDMD or VFA profiles were unaffected by S concentration. Likewise, Smith et al. (2009) observed a linear

increase in H_2S concentration with increasing S content of SFC-based substrate at 0.2, 0.4, and 0.8%, but S concentration had no effect on *in vitro* total gas production, IVDMD or VFA profiles.

Conclusion

Dietary S is an essential mineral for optimal activity of ruminal microorganisms, as well as for growth and metabolism of the animal host. Many agricultural by-products such as DDGS can contain high levels of S, and, when in excess, S can have deleterious effects on health and performance of cattle. Understanding the interactions between dietary S and other nutrients is vital to mitigate its deleterious effects both on ruminal microorganisms and overall well being of feedlot cattle.

References

Beauchamp, R. O., J. S. Bus, J. A. Popp, C. J. Boreiko, and D. A. Andjelkovich. 1984. A critical review of the literature on hydrogen sulfide toxicity. Crit. Rev. Toxicol. 13:25-97.

Bird, P. R. 1972. Sulphur metabolism and excretion studies in ruminants X. Sulphide toxicity in sheep. Aust. J. Biol. Sci. 25:1087-1098.

Bird, P. R. 1974. Sulphur metabolism and excretion studies in ruminants. XIII. Intake and utilization of wheat straw by sheep and cattle. Aust. J. Agric. Res. 25:631-642.

Block, R. J., J. A. Stekol, and J. K. Loosli. 1951. Synthesis of S amino acids from inorganic sulfates by ruminants II. Synthesis of cystine and methionine from sodium sulfate by the goat and by the microorganisms of the rumen of the ewe. Arch. Biochem. Biophys. 33:353–363.

Bolsen, K. K., W. Woods, and T. Klopfenstein. 1973. Effect of methionine and ammonium sulfate upon performance of ruminants fed high corn rations. J. Anim. Sci. 36:1186-1190.

Bray, A. C., and A. R. Till. 1975. Metabolism of sulphur in the gastro-intestinal tract. Pages 243-260 in Digestion and Metabolism in the Ruminant. I.W. McDonald and A. C. I. Warner, ed. Univ. New England Publ. Unit, Armidale.

Bryden, J. M., and A. C. Bray. 1972. The effect of dietary molybdenum on the reduction of inorganic sulphate in the rumen. Proc. Aust. Soc. Anim. Prod. 9:335-340.

Buckner, C. D., T. L. Mader, G. E. Erickson, S. L. Colgan, K. K. Karges, and M. L. Gibson. 2007. Optimum concentrations of dry distillers grains with solubles for finishing beef steers. Beef Cattle Report MP 90. Pages 36-38. University of Nebraska, Lincoln.

Buckner, C.D., M. F. Wilken, J. R. Benton, S. J. Vanness, V. R. Bremer, T. J. Klopfenstein, P. J. Konoff, and G. E. Erickson. 2011. Nutrient variability for distillers grains plus solubles and dry matter determination of ethanol by-products. Prof. Anim. Sci. 27:57-64.

Bulgin, M. S., S. D. Lincoln, and G. Mather. 1996. Elemental sulfur toxicosis in a flock of sheep. J. Am. Vet. Med. Assoc. 208:1063-1065.

Cammack, K. M., C. L. Wright, K. J. Austin, P. S. Johnson, R. R. Cockrum, K. L. Kessler, and K. C. Olson. 2010. Effects of high-sulfur water and clinoptilolite on health and growth performance of steers fed forage-based diets. J. Anim. Sci. 88:1777-1785.

Corrigan, M. E., G. E. Erickson, T. J. Klopfenstein, M. K. Luebbe, K. J. Vander Pol, N. F. Meyer, C. D. Buckner, S. J. Vanness, and K. J. Hanford. 2009. Effect of corn processing method and corn wet distillers grains plus solubles inclusion concentration in finishing steers. J. Anim. Sci. 87: 3351-3362.

Cummings, B. A., D. R. Caldwell, D. H. Gould, and D. W. Hamar. 1995. Identity and interactions of rumen microbes associated with dietary sulfate-induced polioencephalomalacia in cattle. Am. J. Vet. Res. 56:1384-1389.

Dar, S. A., R. Kleerebezem, J. G.Kuenen, and G. Muyzer. 2008. Competition and coexistence of sulfate –reducing bacteria, acetogens and methanogens in a lab-scale anaerobic bioreactor as affected by changing substrate to sulfate ratio. Appl. Microbiol. Biotechnol. 78:1045-1055.

DeClerck, J. C. 2009. Effects of wet corn and sorghum distillers grain on performance carcass characteristics of finishing beef cattle and on *in vitro* fermentation of steam-flaked corn based substrates. M.S. Thesis. Texas Tech Univ., Lubbock.

Dougherty, R. W., and H. M. Cook. 1962. Routes of eructated gas expulsion in cattle – A quantitative study. Am. J. Vet. Res. 23: 997-1000.

Emery, R. S., C. K. Smith, L. Fai To. 1957. Utilization of inorganic sulphate by rumen microorganisms. II. The ability of single strains of rumen bacteria to utilize inorganic sulphate. Appl. Microbiol. 5:363-366.

Gawthorne, J. M., and C. J. Nader. 1976. The effect of molybdenum on the conversion of sulphate to sulphide and microbial-protein-sulphur in the rumen of sheep. Br. J. Nutr. 35:11-23.

Goodrich, R. D. and A. D. Tfllman. 1966. Effects of sulfur and nitrogen sources and copper levels on the metabolism of certain minerals by sheep. J. Anim. Sci. 25:484-491.

Gould, D. H., B. A. Cummings, and D. W. Hamar. 1997. *In vivo* indicators of pathologic ruminal sulfide production in steers with diet-induced polioencephalomalacia. J. Vet. Diagn. Invest. 9:72-76.

Gould, D. H. 1998. Polioencephalomalacia. J. Anim. Sci. 76:309-314.

Hintz, H. F. and D. E. 1964. Effect of selenium, sulphur, and sulphur amino acids on nutritional mucular dystrophy in the lamb. J. Nutr. 82:495-498.

Holt, S. M., and R. H. Pritchard. 2004. Composition and nutritive value of corn co-products from dry milling ethanol plants. Pages 1–7 in SD Beef Rep. BEEF 2004-01. South Dakota Agric. Exp. Stn., Brookings.

Huisingh, J., J. J. McNeill, and G. Matrone. 1974. Sulphate reduction by a *Desulfovibrio* species isolated from sheep rumen. Appl. Microbiol. 28:489-497.

Huisingh, J., D. C. Milholland, and G. Matrone. 1975. Effect of molybdate on sulphide production from methionine and sulphate by ruminal microorganisms of sheep. J. Nutr. 105:1199-1205.

Hume, I. D. and P. R. Bird. 1970. Synthesis of microbial protein in the rumen IV. The influence of the level and form of sulphur. Aust. J. Agric. Res. 21:315-322.

Ivancic, J., and W. P. Weiss. 2001. Effect of dietary sulfur and selenium concentrations on selenium balance of lactating Holstein cows. J. Dairy Sci. 84:225-232.

Kahlon, T. S., J. C. Meiske, and R. D. Goodrich. 1975. S metabolism in ruminants. I. *In vitro* availability of various chemical forms of S. J. Anim. Sci. 41:1147-1153.

Kandylis, K. 1984. The role of sulfur in ruminant nutrition. A Review. Livest. Prod. Sci. 11:611-624.

Kang-Meznarich, J. H. and G. A. Broderick. 1981. Effects of incremental urea supplementation on ruminal ammonia concentration and bacterial protein formation. J. Anim. Sci. 51:422-431.

Kennedy, P. M. and L. P. Milligan. 1978. Quantitative aspects of the transformation of sulphur in sheep. Br. J. Nutr. 39:65-84.

Klopfenstein, T. J., G. E. Erickson, and V. R. Bremer. 2008. Board-invited review: Use of distillers by-products in the beef cattle feeding industry. J. Anim. Sci. 86:1223-1231.

Kung L., A. O. Hession, and J. P.Bracht. 1998. Inhibition of sulfate reduction to sulfide by 9, 10-Anthraquinone in *in vitro* ruminal fermentations. J. Dairy. Sci. 81:2251- 2256.

Kung, L., J. P. Bracht, and J. Y. Tavares. 2000. Effects of various compounds on *in vitro* ruminal fermentation and production of sulfide. Anim. Feed Sci. Technol. 84:69–81.

Leibovich, J., J. T. Vasconcelos, and M. L. Galyean. 2009. Effects of corn processing method in diets containing sorghum wet distillers grains plus solubles on performance and carcass

characteristics of finishing beef cattle an on *in vitro* fermentation of diets. J. Anim. Sci. 87:2124-2132.

Loneragan, G. H. D. H. Gould, J. J. Wagner, F. B. Garry, and M. Thoren. 1997. The effects of varying water sulfate content on H_2S generation and health of feedlot cattle. J. Anim. Sc. 75(Suppl. 1): 272 (Abstr.)

Loneragan, G. H., D. H. Gould, R. J. Callan, C. J. Sigurdson, and D. W. Hamar. 1998. Association of excess sulfur intake and an increase in hydrogen sulfide concentrations in the ruminal gas cap of recently weaned beef calves with polioencephalomalacia. J. Am. Vet. Med. Assoc. 11:1599-1604.

Loneragan, G. H., J. J. Wagner, D. H. Gould, F. B. Garry, and M. A. Thoren. 2001. Effects of water sulfate concentration on performance, water intake, and carcass characteristics of feedlot steers. J. Anim. Sci. 79:2941-2948.

May, L. M., M. J. Quinn, C. D. Reinhardt, L. Murray, M. L. Gibson, K. K. Karges, and J. S. Drouillard. 2009. Effects of dry-rolled or steam-flaked corn finishing diets with or without 25% distillers dried grains on ruminal fermentation and apparent total tract digestion. J. Anim. Sci. 87: 3630-3638.

May, M. L., M. J. Quinn, N. DiLorenzo, D. R. Smith and M. L. Galyean. 2011. Effects of roughage concentration in steam-flaked corn-based diets containing wet distillers grains with solubles on feedlot cattle performance, carcass characteristics, and *in vitro* fermentation. J. Anim. Sci. 89:549–559.

McAllister, M. M., D. H. Gould, M. F. Raisbeck, B. A. Cummings, and G. S. Loneragan. 1997. Evaluation of ruminal sulfide concentrations and seasonal outbreak of polioencephalomalacia in beef cattle in a feedlot. J. Am. Vet. Med. Assoc. 211:1275-1279.

Metaxas M. A. and E. A. Delwiche. 1955. The L-cysteine desulfhydrase of Escherichia coli. J. Bacteriol. 70: 735-737.

Nader, C. J. and D. J. Walker. 1970. Metabolic fate of cysteine and methionine in the rumen digesta. Appl. Microbiol. 20:677-681.

Niles G. A., S. E. Morgan, and W. C. Edwards. 2000. Sulfur-induced polioencephalomalacia in stocker calves. J. Vet. Hum. Toxicol. 42:290-291.

NRC. 1980. Mineral Tolerance of Domestic Animals. Ntl. Acad. Press, Washington, D. C.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th ed. Ntl. Acad. Press,

Washington, D. C.

NRC. 2005. Mineral Tolerance of Animals. 2nd ed. Ntl. Acad. Press, Washington, DC.

Peck, H. D. and T. Lissolo. Assimilatory and dissimilatory sulphate reduction: Enzymology and bioenergetics. Pages 99 –132 in The nitrogen and sulfur cycles. Cambridge University Press. Cambridge, England.

Pendlum, L. C., J. A. Boling, and N. W. Bradley. 1976. Plasma and ruminal constituents and performance of steers fed different nitrogen sources and concentrations of sulfur. J. Anim. Sci. 43:1307-1314.

Qi, K., C. D. Lu, and F. N. Owens. 1993. Sulfate supplementation of growing goats: Effects on performance, acid-base balance, and nutrient digestibilities. J. Anim. Sci. 71:1579-1587.

Quinn, M. J., M. L. May, K. E. Hales, N. DiLorenzo, J. Leibovich, D. R. Smith, and M. L. Galyean. 2009. Effects of ionophores and antibiotics on *in vitro* hydrogen sulfide production, dry matter disappearance, and total gas production in cultures with steam-flaked corn-based substrate with or without added sulfur. J. Anim. Sci. 87:1705–1713.

Raisbeck, M. F. 1982. Is polioencephalomalacia associated with high sulfate diets? J. Am. Vet. Med. Assoc. 180:1303-1305.

Ross, J. G. W. Spears, and J. D. Garlich. 1994a. Dietary electrolyte balance effects on performance and metabolic characteristics in finishing steers. J. Anim. Sci. 72:1600-1607.

Ross, J. G., J. W. Spears, and J. D. Garlich. 1994b. Dietary electrolyte balance effects on performance and metabolic characteristics in growing steers. J. Anim. Sci. 72:1842-1848.

Rumsey, T. S. 1978. Effects of dietary S addition and Synovex- S ear implants on feedlot steers fed an all-concentrate finishing ration. J. Anim. Sci. 46:463-477.

Russell, J. B. 2002. ATP production. Page 45 in Rumen Microbiology and Its Role in Ruminant Nutrition. Dep. of Microbiol., Cornell Univ., Ithaca, NY.

Sarturi J. O., G. E. Erickson, T. J. Klopfenstein, J. T. Vasconcelos, K. Rolfe, and M. G. Dib. 2010. Effects of sulfur content of wet or dry distillers grains in beef cattle finishing diets on intake, ruminal pH, and hydrogen sulfide. J. Anim. Sci. 88(E Suppl. 3):557. (Abstr.)

Short, S. B. and W. C. Edwards. 1989. Sulfur (hydrogen sulfide) toxicosis in cattle. Vet. Human Toxicol. 31:451-453.

Shurson, J., S. Noll, and J. Gohil. 2005. Corn by-product diversity and feeding value to non-ruminants. University of Minnesota. Available: http://www.ddgs.umn.edu/articles-swine/2005-Shurson- %20(MNC)%20Corn%20by-product%20diversity.pdf. Accessed April 2, 2011.

Smith, D. R., N. DiLorenzo, J. Leibovich, M. L. May, M. J. Quinn, J. W. Homm, and M. L. Galyean. 2010. Effects of S and monensin concentrations on *in vitro* dry matter disappearance, hydrogen sulfide production, and volatile fatty acid concentrations in batch culture ruminal fermentations. J. Anim. Sci. 88:1503–1512.

Spears, J. W. 2003. Trace mineral bioavailability in ruminants. J. Nutr. 133:1506S-1509S.

Spears, J. W., K. E. Lloyd, and R. S. Fry. 2011. Tolerance of cattle to high dietary sulfur and effect of dietary cation-anion balance. J. Anim Sci. doi:10.2527/jas.2010-3265.

Spiehs, M. J., M. H. Whitney, and G. C. Shurson. 2002. Nutrient database for distillers dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. J. Anim. Sci. 80:2639–2645.

Stewart, C. S and M. P. Bryant. 1988. The Rumen Bacteria. Pages 21–75 in The Rumen Microbial Ecosystem. Elsevier Science Publishing, New York, NY.

Suttle, N. F. 1974. Effects of organic and inorganic sulfur on the availability of dietary copper to sheep. Br. J. Nutr. 32:559-568.

Thomas, W. E., J. K. Loosli, H. H. Williams and L. A. Maynard. 1951. The utilization of inorganic sulfates and urea nitrogen by lambs. J. Nutr. 43:515-523.

Thompson, L. H., M. B. Wise, R. W. Harvey, and E. R. Barrick. 1972. Starea, urea and sulfur in beef cattle rations. J. Anim. Sci. 35:474-480.

Tucker, W. B., J. F. Hogue, D. F. Watermann, T. S. Siverson, Z. Xin, R. W. Hemken, J. A. Jackson, G. D. Adams, and L. J. Spicer. 1991. Role of sulfur and chloride in the dietary cation-anion balance equation for lactating dairy cattle. J. Anim. Sci. 69:1205-1213.

Vanness, S. J., N. F. Meyer, T. J. Klopfenstein, and G. E. Erickson. 2009. Feedlot incidences of sulfur induced polio and ruminal hydrogen sulfide levels with varying hay level inclusion in byproduct diets. Page 106-107. Proc. of Plains Nutr. Counc. Spring Conf., San Antonio, TX. Texas A&M Ext. Publ. No. AREC 09-18.

Vasconcelos, J. T., and M. L. Galyean. 2007. Nutritional recommendations of feedlot consulting nutritionists: The 2007 Texas Tech University survey. J. Anim. Sci. 85:2772–2781.

Whanger, P. D. and G. Matrone. 1966. Effect of dietary sulfur upon the production and absorption of lactate in sheep. Biochim. Biophys. Acta. 124: 273- 279.

Whanger, P. D. and G. Matrone. 1967. Metabolism of lactic, succinic, and acrylic acids by rumen microorganisms from sheep fed sulfur-adequate and sulfur-deficient diets. Biochim. Biophys. Acta. 136: 27- 35.

Whanger, P. D., O. H. Muth, J. E. Oldfield, and P. H. Weswig. 1969. Influence of sulphur on incidence on white muscle disease in lambs. J. Nutr. 97:553-562.

Whanger, P. D. 1972. Sulphur in ruminant nutrition. Wld. Rev. Nutr. Diet. 15: 225-255.

Widdle, F. 1988. Microbiology and ecology of sulfate reducing bacteria. Page 469 in Biology of Anaerobic Microorganisms. Zehnder AJB ed. Wiley, New York, NY.

Wilken, M. F., M. K. Luebbe, G. E. Erickson, T. J. Klopfenstein, and J. R. Benton. 2009. Effects of feeding high concentrations of byproducts in different combinations to finishing steers. Beef Cattle Report MP 92. Pages 76-78. University of Nebraska, Lincoln.

Zinn, R. A., E. Alvarez, M. Mendez, M. Montaño, E. Ramirez, and Y. Shen. 1997. Influence of dietary sulfur concentration on growth performance and digestive function in feedlot cattle. J. Anim. Sci. 75:1723-1728.

Chapter 2 - Evaluation of sulfur content of dried distillers grains with solubles in finishing diets based on steam-flaked corn or dry-rolled corn[1, 2]

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Abstract

Crossbred yearling steers (n = 80; 406 ± 2.7 kg BW) were used to evaluate effects of S concentration in dried distillers grains with solubles (DDGS) on growth performance, carcass characteristics, and ruminal concentrations of CH_4 and H_2S, in finishing steers fed diets based on steam-flaked corn (SFC) or dry-rolled corn (DRC) and containing 30% DDGS (DM basis) with moderate S (0.42% S, MS) or high S (0.65% S, HS). Treatments consisted of SFC diets containing MS (SFC-MS), SFC diets containing HS (SFC-HS), DRC diets containing MS (DRC-MS), or DRC diets containing HS (DRC-HS). High S was achieved by adding H_2SO_4 to DDGS. Ruminal gas samples were analyzed for concentrations of H_2S and CH_4. Steers were fed once daily in quantities that resulted in traces of residual feed in the bunk the following day for 140 d. No interactions ($P \geq 0.15$) between dietary S concentration and grain processing were observed with respect to growth performance or carcass characteristics. Steers fed HS diets had 8.9% lower DMI ($P < 0.001$) and 12.9% lower ADG ($P = 0.006$) than steers fed diets with MS, but S concentration had no effect on G:F ($P = 0.25$). Cattle fed HS yielded 4.3% lighter HCW ($P = 0.006$) and had 16.2% less KPH ($P = 0.009$) than steers fed MS. Steers fed HS had lower ($P = 0.04$) yield grades than steers fed MS. There were no differences among treatments with respect to dressing percentage, liver abscesses, 12th rib fat thickness, LM area, or USDA quality grades ($P \geq 0.18$). Steers fed SFC had less DMI ($P < 0.001$) than steers fed DRC. Grain processing had no effect ($P > 0.05$) on G:F or carcass characteristics. Cattle fed HS had greater ($P < 0.001$) ruminal concentrations of H_2S than cattle fed MS. Hydrogen sulfide concentration was inversely related ($P \leq 0.01$) to ADG (r = -0.58) and DMI (r = -0.67) in cattle fed SFC, and DMI (r = -0.40) in cattle fed DRC. Feeding DDGS that are high in dietary S may decrease DMI of beef steers and compromise growth performance and carcass characteristics of feedlot cattle.

Key words: dried distillers grains, feedlot performance, grain processing, sulfur

Introduction

Distillers grains with solubles (DGS), a by-product of ethanol production, are being routinely included in finishing diets due to widespread expansion of the fuel ethanol industry. Sulfuric acid is used in ethanol production, and diets containing dried distillers grains with solubles (DDGS) may contain high concentrations of S. Corrigan et al. (2009) observed a linear increase in dietary S as DDGS increased from 0 to 40% (DM basis) of the total diet.

Elevated concentrations of dietary S have deleterious effects on cattle performance and carcass characteristics (Bolsen et al., 1973; Loneragan et al., 1997). Kung et al. (1998) suggested that high dietary sulfate may cause respiratory problems and polioencephalomalacia (PEM). Within the rumen, dietary sulfate is reduced to sulfide by sulfate reducing bacteria, and the sulfides then bind H in the rumen to form H_2S. Hydrogen sulfide is eructated from the rumen and an undetermined amount of H_2S may be aspirated into the lungs. When in excess, aspirated H_2S can cause PEM (Gould, 1998). For example, in a study by Buckner et al. (2007), 6 out of 40 steers fed 50% DDGS (dietary S concentration of 0.60%) in combination with DRC died or showed symptoms related to PEM.

The feeding value of DDGS is less in steam-flaked corn (SFC) diets than dry-rolled corn (DRC) diets (Klopfenstein et al., 2008), and could be due partly to high S concentration of DGS. Cattle fed SFC-based diets yield more H^+ ions compared with those fed DRC-based diets (Barajas and Zinn, 1998; Corona et al., 2006). It is thus conceivable that high dietary S may be more deleterious in SFC diets than in DRC diets because more H^+ are available for H_2S production. Therefore, the objective of this study was to evaluate growth performance, carcass characteristics, and composition of fermentative gasses in the ruminal head space of finishing steers fed SFC- or DRC-based finishing diets containing 30% (DM basis) DDGS and varying concentrations of S.

Materials and Methods

Procedures followed in this study were approved by the Kansas State University Institutional Animal Care and Use Committee protocol no. 2315.

Experimental Design

Crossbred yearling steers (n = 80, 406 ± 2.7 kg initial BW) were used to determine the effects of feeding DDGS containing different S concentrations on growth performance, carcass characteristics, and ruminal concentrations of H_2S and CH_4 in ruminal head space of steers fed diets based on SFC or DRC. The study was conducted as a randomized complete block design with a 2 × 2 factorial arrangement of treatments. Factors consisted of dietary S concentration (moderate S (0.42) or high S (0.65%) of dietary DM; MS and HS, respectively) and grain processing method (SFC or DRC). All diets included 30% DDGS (DM basis) and were based on SFC containing MS (SFC-MS) or HS (SFC-HS), or DRC containing MS (DRC-MS) or HS (DRC-HS). Whole corn was steam-flaked to a bulk density of 360 g/L.

The 0.65% concentration was chosen based on findings by Buckner et al. (2007) that 15% of cattle fed 50% DDGS (dietary S concentration of 0.60%) in a DRC-based diet exhibited signs of S toxicity. The 0.42% (DM basis) MS concentration was made up of the individual S concentrations of ration ingredients (0.13% from corn and other ration ingredients plus 0.29% from DDGS). The 0.65% concentration was attained by mixing 10 kg of H_2SO_4 (93% concentration) with 446 kg of DDGS prior to mixing rations. Consequently, the 0.65% S (DM basis) was comprised of 0.13% S from other ration ingredients, 0.29% S from DDGS, and 0.23% S from added sulfuric acid. Sulfuric acid was used as a S source to mimic actual use in ethanol plants. New loads of DDGS were received once every 6 wk (3 loads for the entire 140-d study period) from Poet Nutrition (Sioux Falls, SD). Sulfur concentration was similar for the 3 loads (0.97% ± 0.01, Table 2.1) and pH for treated DDGS was 2.9, whereas pH for untreated DDGS was 4.5. One metric ton of H_2SO_4-treated DDGS was mixed once weekly.

All finishing diets were formulated to provide (DM basis) 14% CP, 300 mg/d monensin, 90 mg/d tylosin, 2200 IU/kg vitamin A, 0.3% salt, 0.7% Ca, and 0.7% K. Zilpaterol-HCl was fed in all diets at 8.33 mg/kg of diet DM beginning 24 d before the end of the study and withdrawn for 3 d prior to harvest. Composition of finishing diets is summarized in Table 2.2. Sulfur concentrations in the municipal water supply were non-detectable.

Animal Processing, Housing, and Feeding

On arrival at the Kansas State University feedlot, steers were allowed *ad libitum* access to ground alfalfa hay and municipal water. One day after arrival, steers were identified with an ear

tag that displayed a unique number for each study animal. Before initiation of the study, steers were individually weighed and received an estradiol/trenbolone acetate implant (Revalor 200, Intervet, Inc., Millsboro, DE), a topical parasiticide (Phoenectin pour-on, IVX Animal Health, St. Joseph, MO), a 4-way viral vaccine (Bovishield–IV, Pfizer Inc., New York, NY), and a 7-way clostridial vaccine (Fortress – 7, Pfizer, Inc., New York, NY). Steers were grouped in 20 weight blocks and randomly assigned within block to treatments and pens. Steers were transitioned to finishing diets (Table 2.2) through 4 graduated step-up diets that were formulated to allow gradual adaptation to grain. Each step up diet was fed for 5 d. During the step-up period, alfalfa hay was decreased in 10.25% (DM) increments and was replaced by DRC or SFC in increments of 6.25% DM. Inclusion level of DDGS (Poet Nutrition, Sioux Falls, SD) remained the same throughout the step-up period.

Steers were housed (1 per pen) in 4 barns, each containing 20 individual concrete-surfaced pens. Each pen measured 1.5 m × 6 m. Pens were covered with corrugated roofing to provide shade and were equipped with individual feed bunks and water fountains that allowed *ad libitum* access to feed and clean water. Cattle were observed once daily for general health and clinical signs of PEM from trial initiation until harvest, and observations were recorded.

Diets were mixed once daily, weighed, and hand fed to individual animals around 1000 h. Rations were delivered to each pen once daily in quantities that resulted in only traces of residual feed in the bunk the following day. Weights of fresh feed provided were recorded daily, orts were recorded weekly, and cattle were weighed every 14 d.

On d 28 of the study, 1 animal on the SFC-HS diet exhibited symptoms of PEM, including blindness. This animal was removed from the study and treated with dexamethasone. The animal responded by regaining its sight within 12 h. No other animals experienced any health-related problems. Three animals were fed the wrong diet for approximately 1 wk as a result of a clerical error. Data from all 3 mis-fed animals and the sick animal were excluded from the analysis. As a result, by the end of the study, the DRC-MS, DRC-HS, SFC-MS, and SFC-HS treatments had 18, 19, 20, and 19 steers remaining, respectively.

Sampling and Laboratory Analyses

Sulfur concentrations of all feedstuffs and municipal drinking water were analyzed before formulation of the experimental diets. Solid and liquid feedstuffs were analyzed for S content

following official methods 923.01 (AOAC, 1990, minimum detection limit = 5µg/ mL, intraassay CV = 3.3%; interassay CV = 3.8%, Kansas State University Ruminant Nutrition Laboratory) and 31.012 (AOAC, 1984, minimum detection limit = 5µg/ mL, intraassay CV = 3.1%, SDK Laboratories, Hutchinson, KS), respectively.

Feedstuffs were sampled weekly and monthly composited samples were analyzed for DM, starch, NDF, CP, S, and ether extract. Portions of ground samples of feedstuffs were dried in a forced air oven at 105°C overnight to determine DM (Undersander et al., 1993). Starch contents of feedstuffs were determined according to Herrera-Saldana and Huber (1989) with a Technicon Autoanalyzer III (SEAL Analytical, Mequon, WI, minimum detection limit = 5µg/ mL, intraassay CV = 3.0%; interassay CV = 4.5%, Kansas State University Ruminant Nutrition Laboratory) to measure free glucose (Gochman and Schmitz, 1972). Determination of NDF was conducted using an ANKOM fiber analyzer (ANKOM Technology Corp., Fairport, NY) according to Van Soest et al. (1991). Heat-stable α-amylase (ANKOM Technology Corp) was added to remove residual starch from feedstuff samples. Determination of CP was accomplished by measuring N content with a LECO FP–2000 nitrogen analyzer (LECO Corp., St Joseph, MI, minimum detection limit = 48 µg/ g, intraassay CV = 3.2%; interassay CV = 3.7%, Kansas State University Analytical Laboratory) following AOAC (1995) official method 990.03. Ether extract analysis was performed according to AOAC (1995) official method 920.39.

After attempting to harvest ruminal gas samples from ruminally cannulated animals that were being fed the study diets, it was determined that leakage around the ruminal cannulas resulted in large fluctuations in gas composition due to contamination with ambient air. As an alternative, it was opted to collect ruminal gas samples from all intact animals involved in this study via dorsal rumenocentesis. This procedure was approved by the Kansas State University Institutional Animal Care and Use Committee. On d 69, 83, 90, 97, and 104, ruminal gas samples were collected at 0, 4, 8, and 12 h after feeding to monitor concentrations of ruminal gasses. One barn (20 animals) was sampled at each time point such that at the end of 12 h after feeding, all animals would have been sampled. Sampling times were switched among barns on each sampling day so that each barn will be sampled at 0, 4, 8, or 12 h at a given sampling d.

Ruminal gas samples were aspirated by puncturing the ruminal wall via the left *paralumbar fossa* with an 18-gauge, 88.9 mm needle. Aspirated ruminal gas samples were immediately injected into evacuated 30-mL serum bottles fitted with rubber septa (gray butyl

stoppers) and aluminum crimp seals. Concentrations of H_2S and CH_4 were quantified with a gas chromatograph (SRI Instruments, Torrance, CA) equipped with a thermal-conductivity detector, a flame ionization detector, and a gas sampling valve with a 0.5-mL sample loop. Separation was achieved by using a 0.3-cm × 90-cm Haye Sep D packed Teflon column (SRI Instruments, Torrance, CA). Helium was used as the carrier gas, pressure was maintained at 69 KPa, and oven temperature was maintained at 40°C. Samples of gas (10 mL) were transferred from serum bottles to the sample loop with a gas-tight syringe (10 MDF-LL-GT; SGE, Austin, TX).

Carcass Characteristics Data

Steers were harvested on d 140, and final BW (gross BW * 0.96) were determined immediately before cattle were shipped 451 km to a commercial abattoir in Holcomb, KS. Liver abscesses and HCW were recorded the day of harvest. Incidence and severity of liver abscesses were scored according to the Elanco scoring system: 0 = no abscesses, A^- = 1 or 2 small abscesses or abscess scars, A^0 = 2 to 4 small, well-organized abscesses and A^+ = 1 or more large or active abscesses with or without adhesions (Brink et al., 1990). Subcutaneous fat thickness over the 12th rib, KPH, LM area, marbling score, USDA yield grades, and USDA quality grades were determined after a 48-h chill.

Final BW was determined as HCW divided by a dressing percentage of 63.5%, which is the base value used for the grid system under which the cattle were marketed. This adjustment was made to account for differences in gut fill, and carcass trim was minimal for cattle in this study. Average daily gains were computed by subtracting initial live BW from carcass-adjusted final BW, and dividing the result by days on feed (DOF). Gain efficiencies were computed by dividing ADG by daily DMI. Dressed yield was determined as HCW divided by final shrunk BW.

Statistical Analyses

Feed intake over time was analyzed as repeated measures using the Mixed procedure of SAS version 9.1 (SAS Inst. Inc., Cary, NC). Animal was the experimental unit, and the model statement included dietary S concentration, grain processing method, DOF, and all 3- and 2-way interactions. The repeated measures factor was the DOF*h combination because DOF and h were measured on each of the animals. The DOF*h term was broken down into its factorial components in the analysis. The random effect was weight block. Likewise, ruminal gas data

were analyzed as repeated measures using the Mixed procedure, but in this case the model statement included dietary S concentration, grain processing method (G), DOF, time after feeding (h), all 2-way interactions, and S × G × DOF, and S × G × h interactions. The 4-way interaction was left out because not all 4-way combinations had observations, and means were therefore non-estimable. Pearson correlation analysis was also conducted to evaluate relationships between concentrations of H_2S and CH_4 and ADG, DMI, and G:F.

Non-categorical data pertaining to growth performance and carcass characteristics were analyzed using the Mixed procedure of SAS version 9.1. Animal was the experimental unit, and weight block was used as the random effect. The model statement included dietary S concentration, grain processing method, and interaction between dietary S concentration and grain processing method. Treatment means for non-categorical data were determined using LSMEANS procedure of SAS. A $P \leq 0.05$ was declared significant. Data for USDA yield grades and USDA quality grades were analyzed using the Glimmix procedure of SAS version 9.1. Due to low occurrences, data for liver abscesses were analyzed using Chi-square. Treatment means were separated using pairwise comparisons.

Results and Discussion

There was a 3-way interaction ($P = 0.007$) between dietary S concentration, grain processing, and DOF with respect to feed intake (Figure 2.1). Animals fed SFC-HS had less DMI than the other 3 treatment groups from d 14 through d 98 and d 112 through d 126, whereas steers fed DRC-MS had greater DMI from d 14 through d 63 and d 91 through d 140 compared with the other 3 groups ($P < 0.05$). This could be partly attributable to lower pH (2.9) of treated DDGS compared to pH (4.5) of untreated DDGS. There also were dietary S concentration × DOF, and grain processing × DOF interactions ($P < 0.001$), but there was no interaction ($P = 0.85$) between dietary S concentration and grain processing method.

Loneragan et al. (2001) noted a tendency for interaction between drinking water S concentration and DOF with respect to DMI when cattle were fed SFC-based diets and drinking water containing 1 of the following S concentrations (DM basis): 0.18, 0.19, 0.22, 0.29, or 0.40%. Increasing S concentrations tended to depress DMI intake during the first 28 DOF after which feed consumption improved and remained relatively stable until the end of the study. Zinn et al. (1997) observed a linear decrease in DMI when they fed SFC-based diets containing 0.15,

0.20, or 0.25% S (DM). In the present study, HS yielded greater H_2S concentrations than MS, but concentrations of H_2S in the ruminal gas cap decreased with increasing DOF ($P < 0.001$). It is conceivable that the liver was unable to detoxify elevated amounts of sulfide absorbed during early DOF, resulting in high sulfide concentrations in blood (Loneragan et al., 2001).

Treatment effects on ADG and G:F are presented in Table 2.3. Averaged over the entire feeding period, no interactions ($P > 0.25$) between grain processing method and dietary S concentration were observed with respect to growth performance. Feeding HS decreased ADG ($P = 0.006$) and shrunk final BW ($P = 0.009$), but dietary S concentration had no effect ($P = 0.25$) on G:F. Steers fed diets containing HS had 12.9% less ADG ($P < 0.01$ than their counterparts fed MS diets. Dietary S concentration did not affect ($P = 0.50$) dietary NE_m or NE_g calculated on the basis of cattle performance (NRC, 1984).

In agreement with these results, Loneragan et al. (2001) observed decreased ADG and G:F with increasing S content in drinking water. In the Zinn et al. (1997) study, ADG and G:F responded quadratically to increasing dietary S, with the optimal concentration being 0.20%. When evaluating dietary S concentration (0.11, 0.20, 0.28, or 0.38% DM basis) in diets for growing goats, Qi et al. (1993) reported decreased ADG, DMI, and G:F with dietary S greater than 0.20%. However, research by Rumsey (1978) suggested that addition of 0, 0.14, and 0.42% of sublimed S in cracked corn-based diets (0.14% S in basal diet) did not affect ADG but reduced DMI and, as a result, improved feed efficiency of beef steers. The 0.98% S treatment was removed from the study by Rumsey (1978) before the completion of the trial because of a great depression in intake. In a study by Pendlum et al. (1976), feeding 0.11, 0.26, or 0.45% dietary S in ground corn-based diets did not affect growth performance of finishing Holstein steers. Thompson et al. (1972) compared the effects of 0.12 and 0.37% dietary S on performance of steers fed ground corn-based finishing diets in 2 trials. In the first study, increased dietary S had no effect on ADG, but reduced DMI. In trial 2, increasing dietary S depressed ADG and DMI.

In this study and studies by Loneragan et al. (2001), Zinn et al. (1997), and Qi et al. (1993), dietary S was provided mainly in the form of sulfates, whereas in research by Rumsey (1978), Pendlum et al. (1976), and Thompson et al. (1972), elemental S was the main source of dietary S. Therefore, observed differences in response to dietary S may be attributable to differences in bioavailability of S sources. According to Kahlon et al. (1975), elemental S is only

35.8% available for ruminal microbial growth, whereas supplemental S as sulfate is roughly 50% available to ruminal microbes.

Grain processing method had no effect ($P > 0.05$) on ADG or G:F, but steers fed DRC had greater ($P = 0.0001$) DMI than steers fed SFC-based diets. Additionally, diets based on SFC provided more ($P = 0.0001$) NE_m or NE_g than DRC-based diets which is in agreement with previous research that reported the feeding value of SFC to be greater than that of DRC when fed in finishing diets (Barajas and Zinn, 1998; Zinn et al., 1998).

Treatment effects on carcass characteristics of feedlot steers are summarized in Table 2.4. There was no dietary S concentration × grain processing method interaction ($P \geq 0.15$) with respect to carcass characteristics. High dietary S decreased ($P = 0.006$) HCW by 4.3% and KPH fat by 16.2% ($P = 0.009$). Cattle fed HS yielded carcasses with lower ($P = 0.04$) yield grades compared to steers fed diets containing MS. There were no differences among treatments ($P > 0.05$) with respect to dressing percentage; fat thickness over the 12th-rib; LM area; liver abscesses; marbling scores, or USDA quality grades. Grain processing method also had no discernable effect ($P \geq 0.12$) on carcass characteristics.

Similarly, research by Loneragan et al. (2001) and Zinn et al. (1997) indicated decreased HCW with increasing water S content and dietary S concentration, respectively. Yield grades decreased linearly in the study by Loneragan et al. (2001), which is in agreement with results of this study, but were not affected by dietary S concentration in the trial by Zinn et al. (1997). In the Loneragan et al. (2001) study, marbling score and USDA quality grades were not affected by S concentration, but high dietary S tended to decrease dressed yield and to increase LM area linearly, unlike the present results. Loneragan et al. (2001) indicated that reasons for the increase in LM area associated with increasing dietary S were unclear. This unexpected result is contrary to findings by Zinn et al. (1997) and Thompson et al. (1972), who observed a linear decrease in LM area with increasing dietary S concentrations. Zinn et al. (1997) and Thompson et al. (1972) also were uncertain of the reasons for smaller LM area with increasing concentrations of S. In our study, LM area was not affected by dietary S concentrations.

Consistent with findings of this study, research by Zinn et al. (1997), Rumsey (1978), and Thompson et al. (1972) indicated no effect of dietary S on dressing percentage, fat thickness, or marbling scores. But contrary to these results, these researchers found no effect of dietary S on KPH. Differences in KPH results may be attributable to differences in the ranges of S

concentrations evaluated compared to ours. The highest dietary S concentration in studies by Zinn et al. (1997), Rumsey (1978), and Thompson et al. (1972) were (DM basis) 0.25, 0.42, and 0.37%, respectively. Given that these researchers noted a linear decrease in DMI with increasing dietary S concentration, steers fed diets containing 0.65% S had lower energy intake than steers fed diets with 0.42% S. As result, cattle fed HS diets had lower KPH than steer fed MS diets in our trial.

Effects of treatments and time after feeding on gas concentrations in the ruminal gas cap are presented in Table 2.5. Within sampling day, there was no grain processing method × dietary S × time post feeding interaction ($P = 0.38$), but interactions between grain processing method and time after feeding ($P = 0.006$) and between dietary S and time post feeding ($P = 0.005$) occurred. Concentrations of H_2S were similar for steers fed DRC- or SFC-based diets during the first 4 h after feeding, but beginning 8 h after feeding, steers fed DRC-based diets had more H_2S concentration than steers fed SFC-based diets. This difference may reflect differences in DMI or in digestion rate between DRC and SFC. Feeding HS increasingly yielded more ($P < 0.001$) H_2S concentration over 12 h after feeding compared with feeding MS.

Effects of treatments and DOF on concentrations of ruminal gasses in the ruminal head space are presented in Table 2.6. There were no 3-way or 2- way interactions with respect to H_2S concentration ($P > 0.05$). There was a grain processing effect ($P = 0.03$) and a dietary S effect ($P < 0.001$) on ruminal H_2S concentrations. Feeding DRC unexpectedly resulted in greater H_2S concentration compared with feeding SFC. This may have been driven by intake, because dietary S concentration was similar within dietary S concentration treatments. Cattle fed diets containing HS had greater H_2S concentrations in the ruminal gas cap than cattle fed diets containing MS, and H_2S was inversely related ($P \leq 0.01$) to ADG (r = -0.58), DMI (r = -0.67) for steers fed SFC (Table 2.7), and DMI (r = -0.40) for steers fed DRC-based diets (Table 2.8).

A number of factors may explain the deleterious effects of high dietary S intake on growth performance. According to Kandylis (1984) and Bird (1972), ruminal and intestinal motility is decreased when excessive H_2S produced as a result of sulfate reduction in the rumen, hence the decrease in DMI. Research by Loneragan et al. (1997) indicated that cattle consuming water with the highest sulfate concentration had a peak in ruminal sulfide concentration that lasted from approximately 15 to 35 DOF. Following this peak, ruminal sulfide concentration decreased substantially. In this study, in spite of collecting ruminal gasses relatively late in the

study (d 69 to 104) due to logistical reasons a decrease in H_2S over time was observed in animals fed HS (Table 2.6).

These results suggest that cattle are likely to adapt to a chronic exposure to high dietary S, possibly due to development of a more stable combination of assimilatory and dissimilatory activities of sulfate-reducing bacteria (Huisingh et al., 1974). Previous research revealed a swift adaptation of sulfate-reducing bacteria to high ruminal sulfate concentration (Bird and Hume, 1971; Bird and Moir, 1971; Lewis, 1954). Moreover, Bird and Moir (1971) investigated the fate of ruminally or duododenally infused sulfate in sheep. They found that sheep ruminally infused with 6 g/d of S as sulfate stopped eating or drinking, whereas the same amount of S given duodenally did not adversely affect feed consumption.

When sulfides resulting from reduction of sulfate by ruminal bacteria are not absorbed across the rumen wall, they can bind to H^+, form H_2S that is eructated and inhaled, potentially leading to respiratory distress and inflammation (Kandylis, 1984). According to Dougherty and Cook (1962), large proportions of eructated gas enter the lungs, where absorption can occur. Sulfide toxicity is often associated with PEM incidences (Kung, 2000; Gould et al., 1997; Raisbeck, 1982). Symptoms of PEM include increased respiration, decreased feed intake, listlessness, muscular incoordination, progressive blindness, and cerebrocortical necrosis (Gould, 1998).

Distillers grains can contain a substantial amount of S (Buckner et al., 2008), thus causing health problems. Corrigan et al. (2009) observed a linear increase of dietary S as DDGS increased from 0 to 40% (DM basis) of the total diet, and 6% of cattle fed diets consisting mainly of blends of WDGS and wet corn gluten feed (0.47 to 0.59% S) died or were diagnosed with PEM (Wilken et al., 2009). Moreover, in a study by Buckner et al. (2007), 6 out of 40 steers fed 50% DDGS (with 0.60% S) in a DRC-based diet did not finish the study because they died or showed symptoms related to PEM. In the present study, one animal on the SFC-HS diet (0.65% S) showed blindness attributed to PEM. Even though only 1 animal in this study exhibited PEM signs, cattle fed diets with HS had consistently elevated ruminal H_2S concentrations compared with cattle fed diets with MS (Table 2.6). Within sampling day, there was no grain processing × dietary S concentration × time after feeding interaction ($P = 0.09$) or dietary S concentration × time after feeding interaction ($P = 0.55$) with respect to ruminal CH_4 concentration. However, there was a grain processing × time after feeding interaction ($P = 0.003$). Steers fed DRC-based

diets had greater ruminal CH_4 concentration than steers fed SFC-based diets at 4, 8, and 12 h post feeding (Table 2.5).

There was a 3-way interaction (P = 0.04) between dietary S concentration, grain processing method, and DOF, and an interaction (P = 0.05) between dietary S concentration and DOF with respect to ruminal concentration (Table 2.6). There was no grain processing × dietary S interaction (P = 0.14) or dietary S effect (P = 0.08). Zinn et al. (1997) observed a linear decrease in estimated CH_4 production with increasing dietary S. These findings may suggest that a large proportion of fermentative H^+ is used to produce CH_4, as evidenced by comparative ruminal concentrations of the 2 gasses in the present study (Table 2.6).

Feeding DRC-based diets resulted in greater (P = 0.03; Table 6) CH_4 concentration than feeding SFC-based diets across all DOF sampled. These data agree with previous findings (Beauchemin and McGinn, 2005, Moss and Newbold, 2000, Johnson and Johnson, 1995).

Ruminal concentration of CH_4 was negatively correlated (P = 0.01) to DMI (r = - 0.39), ADG (r = - 0.40) for steers fed SFC diets (Table 2.7). Methane concentration also was inversely related ($P \leq 0.05$) to DMI (r = - 0.33), ADG (r = - 0.38) and G:F (r = - 0.33) in steers fed DRC-based diets (Table 2.8).

In conclusion, feeding large amounts of a feed that is high in S, such as DDGS, will contributed to high dietary S and decrease feed intake and reduce energy efficiency of the diet by producing more undesirable H_2S and CH_4. Overall, this could compromise growth performance and health of feedlot cattle.

References

AOAC. 1984. Official Methods of Analysis. 14th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 1990. Official Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 1995. Official Methods of Analysis. 16th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Barajas, R., and R. A. Zinn. 1998. The feeding value of dry-rolled corn and steam-flaked corn in finishing diets for feedlot cattle: Influence of protein supplementation. J. Anim. Sci. 76:1744-1752.

Beauchemin, K. A., and S. M. McGinn. 2005. Methane emissions from feedlot cattle fed barley or corn diets. J. Anim. Sci. 83:653-661.

Bird, P. R. 1972. Sulphur metabolism and excretion studies in ruminants X. Sulphide toxicity in sheep. Aust. J. Biol. Sci. 25:1087-1098.

Bird, P. R., and I. D. Hume. 1971. Sulphur metabolism and excretion studies in ruminants. IV. Cystine and sulphate effects upon the flow of sulphur from the rumen and upon sulphur excretion by sheep. Aust. J. Agric. Res. 22:443-452.

Bird, P. R., and R. J. Moir. 1971. Sulphur metabolism and excretion studies in ruminants. I. The absorption of sulphate in sheep after intraruminal or intraduodenal infusions of sodium sulphate. Aust. J. Biol. Sci. 24:1319-1328.

Bolsen, K. K., W. Woods, and T. Klopfenstein. 1973. Effect of methionine and ammonium sulfate upon performance of ruminants fed high corn rations. J. Anim. Sci. 36:1186-1190.

Brink, D. R., S. R. Lowry, R. A. Stock and J. C. Parrott. 1990. Severity of liver abscesses and efficiency of feed utilization of feedlot cattle. J. Anim. Sci. 68:1201-1207.

Buckner, C. D., S. J. Vanness, G. E. Erickson, T. J. Klopfenstein, and J. R. Benton. 2008. Sampling wet distillers grains plus solubles to determine nutrient variability. Beef Cattle Report MP 91. Pages 126-127. University of Nebraska, Lincoln.

Buckner, C. D., T. L. Mader, G. E. Erickson, S. L. Colgan, K. K. Karges, and M. L. Gibson. 2007. Optimum concentrations of dry distillers grains with solubles for finishing beef steers. Beef Cattle Report MP 90. Pages 36-38. University of Nebraska, Lincoln.

Corona, L. C., F. N. Owens, and R. A. Zinn. 2006. Impact of corn vitreousness and processing on site and extent of digestion by feedlot cattle. J. Anim. Sci. 84:3020-3031.

Corrigan, M. E., G. E. Erickson, T. J. Klopfenstein, M. K. Luebbe, K. J. Vander Pol, N. F. Meyer, C. D. Buckner, S. J. Vanness, and K. J. Hanford. 2009. Effect of corn processing method and corn wet distillers grains plus solubles inclusion concentration in finishing steers. J. Anim. Sci. 87: 3351-3362.

Dougherty, R. W., and H. M. Cook. 1962. Routes of eructated gas expulsion in cattle – A quantitative study. Am. J. Vet. Res. 23: 997-1000.

Gochman, N., and J. M. Schimitz. 1972. Application of new peroxide indicator reaction to specific automated determination of glucose with glucose oxidase. Clin. Chem. 18:943-950.

Gould, D. H., B. A. Cummings, and D. W. Hamar. 1997. *In vivo* indicators of pathologic ruminal sulfide production in steers with diet-induced polioencephalomalacia. J. Vet. Diagn. Invest. 9:72-76.

Gould, D. H. 1998. Polioencephalomalacia. J. Anim. Sci. 76:309-314.

Herrera-Saldana R., and J. T. Huber. 1989. Influence of varying protein and starch degradabilities on performance of lactating cows. J. Dairy Sci. 72:1477-1483.

Huisingh, J., J. J. McNeill, and G. Matrone. 1974. Sulphate reduction by a *Desulfovibrio* species isolated from sheep rumen. Appl. Microbiol. 28:489-497.

Johnson, K. A., and D. E. Johnson. 1995. Methane emissions from cattle. J. Anim. Sci. 73:2483-2492.

Kahlon, T. S., J. C. Meiske, and R. D. Goodrich. 1975. S metabolism in ruminants. I. *In vitro* availability of various chemical forms of S. J. Anim. Sci. 41:1147-1153.

Kandylis, K. 1984. Toxicology of S in ruminants: Review. J. Dairy Sci. 67:2179–2187.

Klopfenstein, T. J., G. E. Erickson, and V. R. Bremer. 2008. Board-invited review Use of Distillers byproducts in the beef cattle feeding industry. J. Anim. Sci. 86:1223:1231.

Kung, L., A. O. Hession, and J. P. Bracht. 1998. Inhibition of sulfate reduction to sulfide by 9, 10-anthraquinone in *in vitro* ruminal fermentations. J. Dairy Sci. 81:2251-2256.

Kung, L., J. P. Bracht, and J. Y. Tavares. 2000. Effects of various compounds on *in vitro* ruminal fermentation and production of sulfide. Anim. Feed Sci. Technol. 84:69–81.

Lewis, D. 1954. The reduction of sulphate in the rumen of the sheep. Biochem. J. 56:391-399.

Loneragan, G. H., D. H. Gould, J. J. Wagner, F. B. Garry, and M. A. Thoren. 1997. The effect of varying water sulfate content on H2S generation and health of feedlot cattle. J. Anim. Sci. 75 (Suppl.1):272. (Abstr.)

Loneragan, G. H., J. J. Wagner, D. H. Gould, F. B. Garry, and M. A. Thoren. 2001. Effects of water sulfate concentration on performance, water intake, and carcass characteristics of feedlot steers. J. Anim. Sci. 79:2941-2948.

Moss, A. R., J. P. Jouany, and J. Newbold. 2000. Methane production by ruminants: Its contribution to global warming. Ann. Zootech. 49:231-253.

NRC. 1984. Nutrient Requirement of Beef Cattle. 6th rev. ed. Natl. Acad. Press, Washington, DC.

Pendlum, L. C., J. A. Boling, and N. W. Bradley. 1976. Plasma and ruminal constituents and performance of steers fed different nitrogen sources and concentrations of sulfur. J. Anim. Sci. 43:1307-314.

Qi, K., C. D. Lu, and F. N. Owens. 1993. Sulfate supplementation of growing goats: Effects on performance, acid-base balance, and nutrient digestibilities. J. Anim. Sci. 71:1579-1587.

Raisbeck, M. F. 1982. Is polioencephalomalacia associated with high sulfate diets? J. Am. Vet. Med. Assoc. 180:1303-1305.

Rumsey, T. S. 1978. Effects of dietary S addition and Synovex- S ear implants on feedlot steers fed an all-concentrate finishing ration. J. Anim. Sci. 46:463-477.

Thompson, L. H., M. B. Wise, R. W. Harvey, and E. R. Barrick. 1972. Starea, urea and sulfur in beef cattle rations. J. Anim. Sci. 35:474-480.

Undersander, D., R. D. Mertens, and N. Thiex. 1993. Forage Analysis Procedures. Natl. Forage Testing Assoc., Omaha, NE.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Wilken, M. F., M. K. Luebbe, G. E. Erickson, T. J. Klopfenstein, and J. R. Benton. 2009. Effects of feeding high concentrations of byproducts in different combinations to finishing steers. Beef Cattle Report MP 92. Pages 76-78. University of Nebraska, Lincoln.

Zinn, R. A., E. Alvarez, M. Mendez, M. Montaño, E. Ramirez, and Y. Shen. 1997. Influence of dietary sulfur concentration on growth performance and digestive function in feedlot cattle. J. Anim. Sci. 75:1723-1728.

Zinn, R. A., E. G. Alvarez, M. F. Montano, A. Plascencia, and J. E. Ramirez. 1998. Influence of tempering on the feeding value of rolled corn in finishing diets for feedlot cattle. J. Anim. Sci. 76:2239-2246.

Table 2.1 Composition of corn dried distillers grains with solubles (DDGS) used in finishing diets[1]

Item, % DM	Moderate S DDGS	High S DDGS[2]
DM	88.9	88.2
OM	94.7	94.6
CP	29.4	29.0
NDF	27.7	26.7
Starch	5.8	5.6
Ether extract	12.0	12.0
Ca	0.26	0.26
P	0.92	0.88
S	0.97	1.70

[1]All reported values are analyzed averages for 8 samples (3 samples for each new load + 5 monthly composite samples)

[2]The High S DDGS was obtained by mixing 10 kg of sulfuric acid (93% concentration) with 446 kg of DDGS to attain 1.70% S

Table 2.2 Composition of finishing diets based on steam-flaked corn or dry-rolled corn containing dried distillers grains with solubles (DDGS) with high S (HS) or moderate S (MS)[1]

	Dry-rolled corn		Steam-flaked corn	
Item	Moderate S	High S	Moderate S	High S
Ingredients, % DM				
Steam-flaked corn	-	-	51.1	50.6
Dry-rolled corn	51.4	50.8	-	-
DDGS with high sulfur	-	30.4	-	30.6
DDGS with moderate sulfur	29.9	-	30.1	-
Alfalfa hay	8.5	8.6	8.6	8.6
Cane molasses	6.2	6.2	6.2	6.2
Supplement[2, 3]	4.0	4.0	4.0	4.0
Analyzed composition, % DM				
DM	87.2	86.6	84.1	83.4
Starch	38.3	38.4	38.8	38.9
CP	15.6	15.4	15.2	15.0
Ether extract	5.8	5.8	5.8	5.8
NDF	12.6	12.2	12.5	12.1
Ca	0.7	0.7	0.7	0.7
P	0.4	0.4	0.4	0.4
K	0.7	0.7	0.7	0.7
S	0.42	0.65	0.42	0.65

[1] All reported values are analyzed averages for 5 monthly composite samples.

[2] Formulated to provide 300 mg/d monensin (Elanco Animal Health, Indianapolis, IN) ; 90 mg/d tylosin (Elanco Animal Health, Indianapolis, IN); 2,200 IU/kg vitamin A; 22 IU/kg vitamin E; 10 mg/kg Cu; 60 mg/kg Zn; 60 mg/kg Mn; 0.5 mg/kg I; 0.25 mg/kg Se; and 0.15 mg/kg Co (DM basis).

[3] Zilpaterol-HCl (Intervet, Inc., Millsboro, DE), was fed for 21 d prior to harvest at 8.33 mg/kg of diet DM, followed by a 3-d withdraw period.

Table 2.3 Growth performance of steers fed finishing diets based on steam-flaked corn or dry-rolled corn containing dried distillers grains with solubles with moderate S (MS) or high S (HS)

	Dry-rolled corn		Steam-flaked corn			*P*-values[1]		
	MS	HS	MS	HS	SEM	G	S	G × S
n	18	19	20	19	-	-	-	-
Days on feed	140	140	140	140	-	-	-	-
Initial BW, kg	412	411	413	412	2.74	0.15	0.43	0.59
Final BW, kg	643	608	627	606	10.9	0.34	0.006	0.46
Shrunk BW, kg	617	591	616	588	10.0	0.64	0.009	0.86
ADG[2], kg	1.64	1.41	1.55	1.37	0.07	0.30	0.006	0.48
DMI, kg/d	10.7	9.8	9.7	8.8	0.27	0.0001	0.0005	0.97
G:F	0.156	0.144	0.158	0.159	0.005	0.07	0.25	0.27
NE_m[3], Mcal/kg	2.28	2.22	2.42	2.41	0.05	0.001	0.50	0.63
NE_g[3], Mcal/kg	1.59	1.54	1.71	1.71	0.04	0.001	0.50	0.61

[1]G = effect of grain processing method; S = effect of sulfur concentration; G × S: interaction between grain processing method and sulfur concentration

[2]Final weight was calculated by dividing carcass weight by a common dressing percentage (63.5%).

[3]Calculated from animal performance using prediction equations from Beef NRC, 1984.

Table 2.4 Carcass performance of steers fed finishing diets based on dry-rolled corn or steam-flaked corn containing dried distillers grains with solubles with moderate S (MS) or high S (HS)

	Dry-rolled corn		Steam-flaked corn			*P*-value[1]		
Item	MS	HS	MS	HS	SEM	G	S	G × S
n	18	19	20	19				
HCW, kg	409	386	398	385	7.3	0.34	0.006	0.45
Dressing, %	66.1	65.4	65.1	65.5	0.40	0.19	0.61	0.15
LM area, cm^2	92.9	92.3	95.5	94.2	2.06	0.19	0.51	0.91
KPH, %	1.88	1.45	1.76	1.60	0.12	0.82	0.009	0.25
12th rib fat, cm	1.62	1.37	1.47	1.02	0.45	0.58	0.82	0.41
Liver abscess[2], %	5.9	0	15.0	0	5.37	-	-	-
Marbling score[3]	394	382	369	403	16	0.91	0.13	0.48
Yield grade	2.34	2.00	2.15	1.79	0.19	0.23	0.04	0.95
Choice, %	41.2	31.6	50.0	31.6	12.0	0.64	0.26	0.64
Select, %	52.9	57.9	25.0	57.9	11.9	0.12	0.18	0.25
Standard, %	5.9	10.5	25.0	10.5	8.3	0.15	0.94	0.40

[1]G = effect of grain processing method; S = effect of sulfur concentration; G × S = interaction between grain processing method and sulfur concentration

[2]Chi-square test = no treatment effect, $P = 0.15$

[3]Marbling score 300 - 399 = Slight

400 - 499 = Small

Table 2.5 Effect of time after feeding on concentrations of ruminal gasses of steers fed finishing diets based on steam-flaked corn or dry-rolled corn containing dried distillers distillers grains with solubles with moderate S (MS) or high S (HS)

	Dry-rolled corn		Steam-flaked corn			*P*-values[1]					
	MS	HS	MS	HS	SEM	G	S	h	G × h	S × h	G × S × h
n	18	19	20	19							
Time after feeding, h	Methane, % of ruminal head space gas										
0	17.6	18.5	18.6	19.0	0.82	0.03	0.08	< 0.001	0.003	0.55	0.09
4	14.4	18.8	13.9	13.4	0.93						
8	15.1	16.4	13.3	14.1	0.93						
12	12.7	14.3	12.1	12.2	0.93						
Time after feeding, h	Hydrogen sulfide, mg/L of ruminal head space gas										
0	1,405	1,612	1,623	1,656	137	0.03	< 0.001	< 0.001	0.006	0.005	0.38
4	2,194	2,887	2,150	2,730	159						
8	2,482	3,509	2,512	2,952	159						
12	3,158	3,760	2,624	3,262	159						

[1]G: effect of grain processing; S: effect of sulfur concentration; h: effect of time after feeding; G × h: interaction between grain processing method and time after feeding; S × h: interaction between sulfur concentration and time after feeding; G × S × h: 3-way interaction between grain processing method, sulfur concentration, and time after feeding

Table 2.6 Effect of days on feed on concentrations of ruminal gasses of steers fed finishing diets based on steam-flaked corn or dry-rolled corn containing dried distillers grains with solubles with moderate sulfur (MS) or high sulfur (HS)

	Dry-rolled corn		Steam-flaked corn			*P*-values[1]						
	MS	HS	MS	HS	SEM	G	S	G × S	DOF	G × DOF	S × DOF	G × S × DOF
n	18	19	20	19								
Days on feed[2]	Methane, % of ruminal head space gas											
69	9.6[b]	15.0[a]	9.2[b]	9.3[b]	1.09	0.03	0.08	0.14	< 0.001	0.08	0.05	0.04
83	16.5[a]	17.4[a]	13.7[b]	15.9[a]	0.91							
90	15.6	17.0	14.9	15.2	0.91							
97	17.0	18.5	18.3	16.5	0.91							
104	16.0	17.2	16.2	16.4	0.91							
Days on feed[2]	Hydrogen sulfide, mg/L of ruminal head space gas											
69	2,420	2,787	2,460	3,064	191	0.03	< 0.001	0.22	< 0.001	0.07	0.11	0.56
83	2,585	3,381	2,334	2,682	155							
90	2,304	2,798	2,288	2,555	155							
97	1,961	2,880	1,815	2,542	155							
104	2,280	2,879	2,243	2,413	155							

[1]G: effect of grain processing method; S: effect of sulfur concentration; DOF: effect of days on feed; G × S: interaction between grain processing method and sulfur concentration; G × DOF: interaction between grain processing method and days on feed; S × DOF: interaction between sulfur concentration and days on feed; G × S × DOF: 3- way interaction between grain processing method, sulfur concentration, and days on feed

[2]Within rows, superscripts with different letters are different ($P < 0.05$)

Table 2.7 Correlation coefficients between concentrations of ruminal head space gasses and performance parameters of steers fed finishing diets based on steam-flaked corn containing dried distillers grains with solubles with moderate S or high S concntrations[1]

Item	CH_4	H_2S	ADG	DMI
H_2S, mg/kg	0.13			
P-value	0.44			
ADG, kg/d	-0.40	-0.58		
P-value	0.01	0.0001		
DMI, kg/d	-0.39	-0.67	0.85	
P-value	0.01	<0.0001	<0.0001	
G:F	-0.31	-0.21	0.77	0.34
P-value	0.06	0.20	<0.0001	0.03

[1]Data used for correlation analysis were collected at the same time

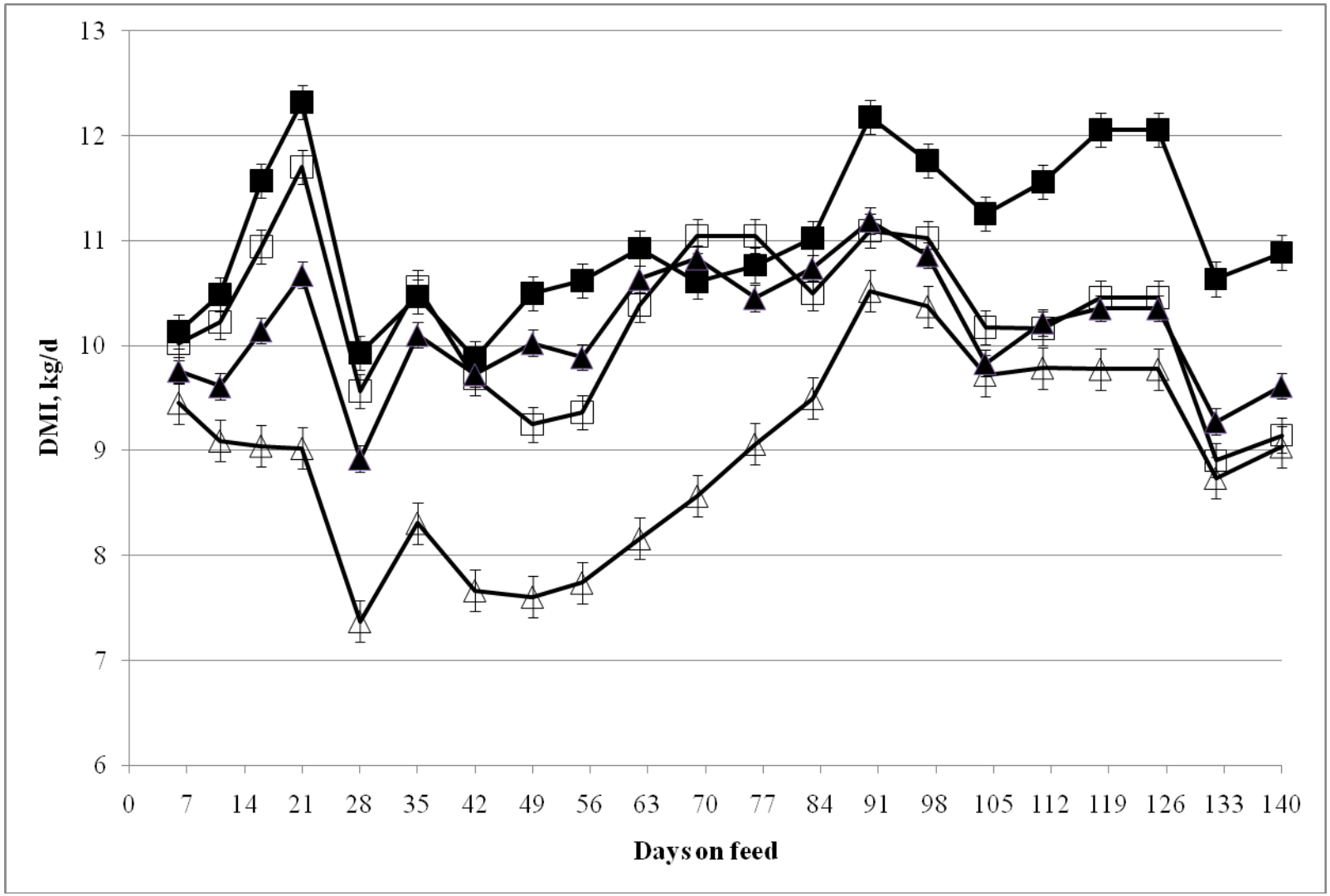
Table 2.8 Correlation coefficients between concentrations of ruminal head space gasses and performance parameters of steers fed finishing diets based on dry-rolled corn containing dried distillers grains with solubles with moderate S or high S concentrations[1]

Item	CH_4	H_2S	ADG	DMI
H_2S, mg/L	0.19			
P-value	0.25			
ADG, kg/d	-0.38	-0.26		
P-value	0.02	0.11		
DMI, kg/d	-0.33	-0.40	0.81	
P-value	0.05	0.01	<0.0001	
G:F	-0.33	-0.08	0.81	0.33
P-value	0.05	0.65	<0.0001	0.04

[1]Data used for correlation analysis were collected at the same time

Figure 2.1 Dry matter intake by crossbred steers fed finishing diets based on steam-flaked corn (SFC, triangles) or dry-rolled corn (DRC, squares) containing dried distillers grains with moderate S (MS, solid symbols) or high S (HS, open symbols).

The number of observations was 18, 19, 20, and 19 for DRC with MS; DRC with HS; SFC with MS; and SFC with HS, respectively. There was a 3-way interaction ($P = 0.007$) between grain processing method, S concentration, and days on feed. There were also interactions between S concentration and days on feed ($P < 0.001$); and grain processing method and days on feed ($P < 0.001$).


13
12
11
10
9
8
7
6
DMI, kg/d
0
7
14
21
28
35
42
49
56
63
70
77
84
91
98
105
112
119
126
133
140
Days on feed

Chapter 3 - Effects of distillers grains with high sulfur concentration on ruminal fermentation and digestibility of finishing diets[1, 2]

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Abstract

Twelve ruminally cannulated crossbred Angus steers were used to evaluate ruminal fermentation characteristics and diet digestibility when 30% (DM) corn dried distillers grains with solubles (DDGS) containing 0.42 or 0.65% (DM) of dietary S was incorporated into finishing diets based on steam-flaked corn (SFC) or dry-rolled corn (DRC). The study was a replicated, balanced randomized incomplete block design with a 2 × 2 factorial arrangement of treatments. Factors consisted of dietary S concentration (0.42 and 0.65% of DM; 0.42S and 0.65S, respectively) and grain processing method (SFC or DRC). The 0.65S concentration was achieved by adding H_2SO_4 to DDGS prior to mixing rations. Steers were assigned randomly to diets and individual, slatted-floor pens, and fed once daily for *ad libitum* intake. Two 15-d experimental periods were used, each consisting of a 12-d diet adaptation phase and a 3-d sample collection phase. Samples were collected at 2-h intervals post-feeding during the collection phase. Ruminal pH was measured immediately after sampling, and concentrations of ruminal ammonia and VFA were determined. Fecal samples were composited by steer within period and used to determine apparent total tract digestibilities of DM, OM, NDF, CP, starch, and ether extract. Feeding 0.65S tended ($P = 0.08$) to decrease DM intake but resulted in greater apparent total tract digestibilities of DM ($P = 0.04$) and ether extract ($P = 0.03$). Ruminal pH increased ($P < 0.05$) in steers fed 0.65S diets, which may be attributable, in part, to decreased ($P = 0.05$) VFA concentrations and greater ($P < 0.01$) ruminal ammonia concentrations when 0.65S was fed, compared to feeding 0.42S. These effects were more exaggerated in steers fed DRC (interaction, $P < 0.01$), compared to steers fed SFC. Steers fed DRC-0.65S had greater ($P < 0.01$) acetate concentration than steers fed DRC-0.42S, but acetate concentration was not affected by S concentration when SFC was fed. Propionate concentration was decreased ($P < 0.01$) in steers fed SFC-0.65S compared to steers fed SFC-0.42S, but dietary S concentration had no effect on propionate concentration when DRC was fed. Butyrate concentration was lesser ($P < 0.01$) in steers fed 0.65S diets than in steers fed 0.42S. Lactate concentrations tended ($P = 0.06$) to decrease in steers fed 0.65S diets. Feeding DDGS with elevated S concentration may decrease feed intake and ruminal VFA concentration but increase ruminal ammonia concentration.

Key words: digestibility, distillers grains, feedlot steers, grain processing, ruminal fermentation, sulfur

Introduction

Distillers grains with solubles (DGS) are becoming increasingly important staples of feedlot cattle diets because of widespread expansion of the fuel ethanol industry. Sulfuric acid is added to fermenters to adjust pH for optimum ethanol yield. Sulfur concentration has been reported to vary from 0.33 to 0.74% DM among dried DGS (Spiehs et al., 2002; Holt and Pritchard, 2004), and 0.36 - 0.39% for wet DGS (Holt and Pritchard, 2004). Within the rumen, sulfate-reducing bacteria produce H_2S from dietary sulfates. Generated sulfides are absorbed from the rumen or can be eructated and inhaled through the lungs, potentially causing respiratory problems (Kandylis, 1984) and polioencephalomalacia (Gould, 1998). In a study by Buckner et al. (2007), 6 out of 40 steers fed 50% dried distillers grains with solubles (DDGS) in dry-rolled corn (DRC) diets (0.60% dietary S concentration) showed symptoms related to S toxicity before the end of the study.

Moreover, a metabolism study by Zinn et al. (1997) indicated that dietary S concentration affected site and extent of fiber and protein digestion. A number of studies evaluated effects of S concentration on ruminal microbial fermentation *in vitro* (Kung et al., 1998; May et al.; 2010, Smith et al., 2010). However, information about effects of DGS containing elevated S concentration on ruminal fermentation and diet digestibility *in vivo* is still insufficient (Sarturi et al., 2010; Nichols et al., 2011). The objective of this study was to investigate ruminal fermentation characteristics and apparent total tract digestibilities in steers fed DRC or steam-flaked corn (SFC) diets that included DDGS with 0.42 or 0.65% S (DM basis). We hypothesized that elevated dietary S concentration may be more deleterious in SFC diets than in DRC diets because high ruminal [H^+] in steers fed SFC-based diets would lead to increased H_2S production compared to feeding DRC-based diets (Gould, 2000; Corona et al., 2006).

Materials and Methods

Procedures followed in this study were approved by the Kansas State University Institutional Animal Care and Use Committee protocol no. 2535.

Crossbred Angus steers (n = 12, 600 ± 12 kg BW) fitted with ruminal cannulas (Bar Diamond Inc., Parma, ID; dorsal sac) were used in a metabolism trial. Steers were housed in individual, slatted-floor pens (1.5 × 3.5 m) equipped with individual feed bunks and water fountains that allowed access to feed and clean water *ad libitum*.

The study was a replicated, balanced randomized incomplete block design with a 2 × 2 factorial arrangement of treatments. The experiment was conducted in two 15-d periods and experimental period was used as the blocking factor. Factors consisted of dietary S concentration (0.42 or 0.65% S of DM; 0.42S or 0.65S, respectively) and grain processing method (DRC or SFC processed to a bulk density of 360 g/L). Starch availability determined using the Kansas State University refractive index method (Sindt et al., 2000) for SFC-based diets was 51.4 ± 4.05%.

All diets included 30% DDGS (DM basis) and were based on SFC containing 0.42S (SFC-0.42S) or 0.65S (SFC-0.65S) or DRC containing 0.42S (DRC-0.42S) or 0.65S (DRC-0.65S). The 0.42S concentration consisted of the individual S contents of ration ingredients whereas 10 kg of H_2SO_4 (93% concentration) were mixed with 446 kg of DDGS prior to mixing the resulting acid-treated DDGS with other ingredients to attain the 0.65S concentration. Sulfuric acid was used as S source to emulate standard commercial procedures during ethanol and DDGS production. The 0.65S concentration was chosen on the basis of a study by Buckner et al. (2007) in which 15% of steers fed DRC-based diets with 50% DDGS (0.60% dietary S concentration) exhibited signs of S toxicity. In the current study 30% DDGS was fed, instead of 50% DDGS (Buckner et al., 2007), to limit deleterious interactions between DDGS and SFC on cattle performance (Klopfenstein et al., 2008) and ruminal microbial fermentation (May et al., 2009). We were interested more in dietary S content in the study by Buckner et al. (2007) than in DDGS inclusion level.

Before formulation of the experimental diets, solid and liquid feedstuffs were analyzed for S content following AOAC (1990) official method 923.01 and AOAC (1984) official method 31.012, respectively. Sulfur concentrations in the municipal water supply were non-detectable, whereas DDGS contained 0.97 ± 0.01% S (DM basis, Table 3.1). Composition of finishing diets fed to cannulated steers is presented in Table 3.2. Diets were mixed in a stationary ribbon mixer (Davis Manufacturing Co., Bonner Springs, KS) approximately 1 h prior to delivery, weighed to the nearest 0.2 kg into individual feed tubs, and delivered once daily at 0800 h. The amount of fresh feed offered to each steer resulted in only traces of unconsumed feed the following day. Each morning before feeding, residual feed was removed, weighed, and subsequently dried at 55°C until a constant weight was obtained (96 h). The weight of orts was subtracted from total feed delivered to estimate DMI.

Each of the two 15-d experimental periods consisted of 12 d for diet adaptation and 3 d for sample collection. Steers were assigned randomly to treatments in period one. Steers were assigned to treatments in period 2 such that each combination of treatments was represented among the 2 periods to complete the balanced block design. This resulted in a total of 6 replicates per treatment. One steer on the SFC-0.65S diet stopped eating and was removed from 2 periods of study. As a result, SFC-0.65S and DRC-0.65S treatments had five observations per treatment at the end of the trial.

Beginning on d 6 (7 d before the 3-d sample collection phase) of each period, chromic oxide (10 g) in gelatin capsules (Torpac Inc., Fairfield, NJ) was placed into the rumen each day before feeding as an indigestible marker to estimate total fecal output. Starting on d 13 of each period, ruminal digesta (approximately 1000 mL) were collected via the ruminal cannula at the following times for each period: d 1 at 0, 6, 12, and 18 h; d 2 at 2, 8, 14, and 20 h; and d 3 at 4, 10, 16, and 22 h relative to feeding. At each sampling point, ruminal fluid samples were strained through 8 layers of cheesecloth, and pH of strained ruminal fluid was recorded immediately with a portable pH meter (model 230, Thermo Orion, Waltham, MA). Strained ruminal fluid (4 mL) was mixed with 1 mL of 25% (wt/vol) metaphosphoric acid and frozen at -20°C for later analyses of ruminal concentrations of VFA, lactate, and ammonia.

Acidified ruminal fluid samples were later thawed to room temperature and centrifuged at 30,000 × g for 20 min, and 1 mL of the supernatant fluid was analyzed for VFA and lactate concentrations by gas chromatography (5890A; Hewlett-Packard, Palo Alto, CA; 2-m × 2-mm column; Carbopack BDA 80/120 4% CW 20M column packing, Supelco Bellefonte, PA) with He as the carrier gas, a flow rate of 24 mL/min, and a column temperature of 175°C. Lactate and the following VFA were analyzed for each sampling time: acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate. Total VFA concentration was computed as the sum of individual VFA concentrations. A portion (1 mL) of the supernatant fluid for each sampling point was analyzed for ruminal ammonia concentration with an Autoanalyzer III (SEAL Analytical, Mequon, WI) following procedures described by Broderick and Kang (1980).

Fecal samples (approximately 500 g on a wet basis) were taken from each steer at the same time as ruminal digesta sample collection. Feedstuff samples and each fecal sample were oven dried at 55°C for 96 h, equilibrated with ambient air, ground through a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass a 1-mm screen, and then placed into plastic bags for long-

term storage. For each steer within each collection period, 5 g of dried, ground fecal sample from each sampling point were pooled, thoroughly homogenized, and subsequently used for determination of chemical composition.

Chemical components analyzed included DM, OM, starch, NDF, CP, and ether extract. Portions of ground samples of feedstuffs and composited feces were dried in a forced-air oven at 105°C overnight and combusted at 450°C for 8 h to determine DM and OM, respectively (Undersander et al., 1993). Starch contents of diets and fecal samples were determined according to Herrera-Saldana and Huber (1989) by using an Autoanalyzer III (SEAL Analytical, Mequon, WI) to measure free glucose (Gochman and Schmitz, 1972).

Determination of NDF was conducted with an ANKOM fiber analyzer (ANKOM Technology Corp., Fairport, NY) according to Van Soest et al. (1991). Heat-stable α-amylase (ANKOM Technology Corp., Fairport, NY) was added to remove residual starch from feedstuff samples before NDF analysis. Determination of CP was accomplished by measuring N content with a N analyzer (FP–2000; LECO Corp., St Joseph, MI) following AOAC (1995) official method 990.03. Ether extract analysis was performed according to AOAC (1995) official method 920.39. Chromium concentrations in fecal samples were measured by using an atomic absorption spectrophotometer with an acetylene/air flame (Perkin Elmer 3110; Norwalk, CT) following procedures described by Williams et al. (1962).

Fecal output for each steer within each period was calculated by dividing chromium dosed by the concentration of chromium in feces (DM basis; assuming that chromic oxide is 100% indigestible). Total tract digestibilities of nutrients by each steer within period were obtained by dividing the difference between nutrient intake (DM basis) and fecal output (DM basis) by nutrient intake.

Apparent total tract digestibilities were analyzed using the mixed model (version 9.1 of SAS; SAS Inst., Cary, NC). Steer was the experimental unit, and period (block) and steer were included as random effects. The model statement included effects of dietary S concentration, grain processing method, and the interaction between dietary S concentration and grain processing method. Ruminal pH and ruminal concentrations of ammonia, VFA, and lactate were analyzed as repeated measures using mixed procedure of SAS. The statistical model consisted of fixed effects of dietary S concentration, grain processing method, time post-feeding within day, and all 2- and 3-way interactions, random effects of steer and experimental period, and the

residual term which was the random error of hourly measurements with steer and period. Treatment means were determined using the LSMEANS option and separated using F-test-protected LSD ($P \leq 0.05$).

Results and Discussion

There was no interaction between grain processing method and dietary S concentration ($P > 0.20$) with respect to nutrient intake (Table 3.3) or excretion thereof. Feeding 0.65S decreased NDF intake ($P = 0.04$) and tended to be associated with lesser intake of DM and OM ($P = 0.08$), CP ($P = 0.06$), starch ($P = 0.08$), and ether extract ($P = 0.08$) compared with feeding 0.42S. Steers fed 0.65S excreted lesser fecal DM ($P = 0.04$), OM ($P = 0.05$), CP ($P = 0.04$), NDF ($P = 0.04$), and ether extract ($P = 0.01$) than steers fed diets with 0.42S.

No grain processing method × dietary S concentration interaction occurred ($P > 0.10$) with respect to nutrient digestibilities (Table3.3). Steers fed 0.65S had greater apparent total tract digestibilities of DM ($P = 0.04$) and ether extract ($P = 0.03$), and tended to have greater apparent total tract digestibility of OM ($P = 0.08$) than steers fed 0.42S. Dietary S concentration had no effect ($P > 0.10$) on apparent total tract digestibilities of other nutrients. The actual amounts of nutrients digested (kg/d) were not affected ($P > 0.10$) by dietary S concentration, suggesting that differences in apparent total tract digestibilities were driven by intake. Steers fed 0.65S diets tended to consume less feed and, consequently, had a greater percentage digestibility than steers fed 0.42S diets (Bird, 1972)**.**

Dietary S concentration did not affect intake or apparent total tract digestion of DM when Boila and Golfman (1991) evaluated effects of 0.12 and 0.39% S on digestion characteristics in Holstein steers fed a barley-based finishing diet. However, Boila and Golfman (1991) reported that steers fed 0.12% S had greater intake of OM and CP than steers fed 0.39% S, but OM and CP total tract digestibilities were unaffected by dietary S concentration. A metabolism study by Zinn et al. (1997) reported no effect of dietary S concentrations (0.15, 0.20, and 0.25% DM) on intake or apparent total tract digestion of DM, OM, N, or starch by steers. Differences between nutrient apparent total tract digestibilities reported herein and those reported by Boila and Golfman (1991) and Zinn et al. (1997) could be attributable to S concentrations evaluated. In studies by Boila and Golfman (1991) and Zinn et al. (1997), S concentrations evaluated were within the limits recommended by NRC (2000) whereas we evaluated S

concentrations at or above recommended dietary concentrations (NRC, 2000). Elevated S concentrations have been reported to decrease ruminal motility due to excessive H_2S produced as a result of sulfate reduction in the rumen, hence a decrease in DMI and increase in apparent total tract digestibility (Bird, 1972). We previously reported negative correlations between ruminal H_2S concentrations and DMI by steers fed finishing diets with elevated S content (Uwituze et al., 2011). In a study by Sarturi et al. (2010), steers fed DDGS containing 1.16% S had less DMI than steers fed DDGS with 0.82% S, and the former tended to have more H_2S concentration than the latter. Increasing S content of substrates from 0.2, 0.4, and 0.8% S had no effect on IVDMD or VFA profiles but increased H_2S production (Smith et al., 2010). Lack of S effect on IVDMD or VFA profiles in vitro may suggest that excessive ruminal H_2S concentration inhibits ruminal motility leading to decreased DMI and improved digestibility in vivo as previously mentioned.

Grain processing method had no effect on nutrient intake ($P > 0.40$; Table 3.3) or fecal excretions of DM, OM, NDF, CP, and ether extract ($P > 0.15$). But as expected, feeding SFC resulted in decreased ($P = 0.03$) starch excretion compared to feeding DRC. Similar to our results, Zinn (1990), Corona et al. (2006), and May et al. (2009) observed decreased starch excretion by steers fed SFC compared with steers fed DRC.

Steers fed SFC diets had greater apparent total tract digestibilities of DM ($P = 0.03$) and starch ($P = 0.02$) and tended to have greater digestibilities of OM ($P = 0.06$) and ether extract ($P = 0.08$) than steers fed DRC diets, but grain processing method had no effects on apparent total tract digestibilities of NDF ($P = 0.37$) or CP ($P = 0.13$; Table 3). Improvements in total tract digestion of DM and starch caused by steam flaking have been reported previously (Zinn, 1990; Barajas and Zinn, 1998; May et al., 2009). Steam-flaking changes structure of starch granule, which makes flaked grains more digestible than when particle size is decreased by dry rolling (Theurer, 1986).

No time post-feeding × dietary S concentration × grain processing method interaction occurred ($P > 0.10$) with respect to all of ruminal fermentation parameters investigated. Effect of time post-feeding was observed for ruminal concentrations of ammonia ($P < 0.01$), acetate ($P < 0.01$), propionate ($P < 0.01$), butyrate ($P = 0.01$), isobutyrate ($P < 0.01$), isovalerate ($P < 0.01$), total VFA ($P < 0.01$, and ruminal pH ($P < 0.01$). Within sampling day, there were 6-h intervals of high and low peaks of these fermentation products. This may indicate that steers consumed large meals in frequent intervals.

Feeding 0.65S yielded greater ($P < 0.01$) ruminal ammonia concentration compared to feeding 0.42S, and these effects were more exaggerated in steers fed DRC (interaction, $P < 0.01$) compared to steers fed SFC (Figure 3.1). Average ruminal ammonia concentrations for DRC-0.42S, DRC-0.65S, SFC-0.42S, and SFC-0.65S were 4.97, 11.06, 3.92, and 6.09 mM, respectively. Microbial metabolism of N and S are interdependent in the rumen. Sulfide has been reported to be the major source of S for bacterial protein synthesis (Kandylis, 1984) whereas ammonia is a source of nonprotein N for microbial protein synthesis (Bach et al., 2005). For every 3.8 mg/L of ruminal sulfide, 85 mg/L of ruminal ammonia is required for optimal ruminal microbial efficiency (Kang-Meznarich and Broderick, 1981; Kandylis, 1984). In the current study, N:S ratios were 5.5 and 3.7 for diets containing 0.42S and 0.65S respectively. Ammonia concentration was least in steers fed SFC-0.42S and greatest in steers fed DRC-0.65S. Because experimental diets were isonitrogenous (Table 3.2), this interaction may suggest that feeding 0.65S yielded less microbial protein (Hume and Bird, 1970), resulting in greater ammonia concentration in steers fed DRC diets compared with steers fed SFC diets. According to Cooper et al. (2002), steers fed SFC diets have greater requirements for degradable intake protein than steers fed DRC diets.

With respect to acetate concentrations, there was an interaction ($P = 0.02$) between grain processing method and dietary S concentration. Steers fed DRC-0.65S had greater acetate concentration than steers fed DRC-0.42S, but acetate concentration was not affected by S concentration when SFC was fed (Figure 3.2). On the other hand, propionate concentration was reduced in steers fed SFC-0.65S compared to steers fed SFC-0.42S, but dietary S concentration had no effect on propionate concentration when DRC was fed (interaction, $P < 0.01$; Figure 3.3). Ruminal acetate concentrations averaged 40.0, 44.3, 38.9, and 37.5 m*M* for DRC-0.42S, DRC-0.65S, SFC-0.42S, and SFC-0.65S, respectively, and average propionate concentrations for DRC-0.42S, DRC-0.65S, SFC-0.42S, and SFC-0.65S were 32.4, 33.1, 35.0, and 26.2 m*M*, respectively. There was no interaction between dietary S concentration and grain processing method for (A:P) ratio ($P = 0.45$). Steers fed 0.65S diets had a greater ($P < 0.001$) A:P ratio than steers fed 0.42S diets. No effect of time post-feeding occurred ($P = 0.16$) for A:P ratio with average values being 1.31; 1.65; 1.28, and 1.53 for DRC-0.42S, DRC-0.65S, SFC-0.42S, and SFC-0.65S, respectively.

Zinn et al. (1997) found that increasing S concentration in SFC-based diets decreased molar proportions of acetate (quadratic effect, $P < 0.10$), and increased molar proportions of propionate (linear effect, $P < 0.10$). Zinn et al. (1997) also reported very small change in these variables when S content increased from 0.15 to 0.20% whereas the major change occurred when S content further increased to 0.25% S. Thompson et al. (1972) observed a similar decrease in ruminal acetate and increase in ruminal propionate in feedlot steers when dietary S was increased from 0.12 to 0.37% in ground corn-based diets. Thompson et al. (1972) also reported a decreased A:P ratio in ruminal fluid of steers fed diets with 0.37% S compared with steers fed 0.12% S.

Differences observed between results reported in the present study and those by Zinn et al. (1997) and Thompson et al. (1972) could be attributable to S concentrations evaluated. Zinn et al. (1997) and Thompson et al. (1972) fed levels that were within the recommended S concentrations (NRC, 2000), whereas concentrations above those recommended were fed in current study. It has been suggested that dietary S increases propionate production by converting lactate to acrylyl-CoA, a S-containing intermediate (Russell, 2002), through acrylate pathway (Whanger and Matrone, 1967). Dietary S concentration also can change dynamics of ruminal microbial population. Although Cummings et al. (1995a) noted no changes in relative counts of sulfate-reducing bacteria, they reported reduction in microbial diversity in ruminal fluid from steers fed diets with added sulfate compared to steers fed diets without added sulfate. Using fluorescence *in situ* hybridization, Dar et al. (2008) reported different bacterial profiles as a result of various lactate to sulfate molar ratios. At greater sulfate concentration sulfate-reducing bacteria that partially oxidize lactate to propionate were predominant, whereas acetogens and methanogenic archea were predominant at low sulfate concentration.

Moreover, elevated sulfate concentration causes sulfate-reducing bacteria that partially oxidize lactate to thrive on fermentation products such as lactate, ethanol, and propionate (Widdle, 1988). This could explain the swift adaptation of sulfate-reducing bacteria to increased ruminal sulfate concentration (Bird and Hume, 1971; Cummings et al., 1995b), which could potentially affect ruminal propionate concentration. Within the rumen, dietary sulfate is reduced to sulfide by sulfate reducing bacteria, and produced sulfide binds H^+ in the rumen to form H_2S (Kandylis, 1984). Propionate and HS^- are both H^+ sinks and the two could compete for H^+ in the rumen (Dar et al., 2008).

Interactions between dietary S concentrations and grain processing method observed for concentrations of acetate and propionate could be attributable to relative concentrations of lactate and sulfate. Whanger and Matrone (1966) reported accumulation of lactate in the rumen of sheep fed S deficient diets whereas lactate was metabolized to VFA in the rumen of sheep whose dietary S requirements were satisfied. According to Dar et al. (2008), at a smaller lactate to sulfate molar ratio, lactate is primarily converted to propionate and acetate to a lesser degree.

In the current study, there tended to be effects of dietary S concentration with respect to ruminal lactate concentration ($P = 0.06$; Figure 3.4). Steers fed 0.65S tended to have a decreased concentration of ruminal lactate concentrations than steers fed 0.42S. Steers fed SFC-0.42S tended ($P = 0.09$) to have more lactate concentrations than other treatments from 8 – 10 h. In a study by May et al. (2009), steers fed SFC-based diets had more lactate concentration than steers fed DRC-based diets between 2 and 16 h after feeding. Moreover, it has been reported that increasing dietary S to correct for S deficiency promotes lactate metabolism to VFA (Whanger and Matrone, 1966; Dar et al., 2008). Thus, the spike in lactate concentration observed in steers fed SFC-0.42S may indicate more lactate was produced than it was metabolized between 8 and 10 h. Lactate concentrations averaged 0.59, 0.35, 1.39, and 0.63 mM, for DRC-0.42S, DRC-0.65S, SFC-0.42S, and SFC-0.65S, respectively.

Zinn et al. (1997) reported that increasing S from 0.15 to 0.25% DM did not influence ($P > 0.10$) lactate concentration. Rumsey (1978) observed a reduction in ruminal lactic acid concentrations in steers fed 0.28 rather than 0.14% dietary S. However, in the Rumsey (1978) study, steers were deprived of feed for 96 h before experimental diets were fed. Qi et al. (1993) did not observe an effect of dietary S concentration on ruminal L-lactic acid concentrations in growing goats fed diets containing 0.11, 0.20, 0.28, or 0.38% S of diet DM.

No interaction between dietary S concentration and grain processing method occurred with respect to ruminal concentrations of butyrate ($P = 0.90$; Figure 3.5), isobutyrate ($P = 0.23$; Table 3.4), or valerate ($P = 0.12$; Table 3.4). Steers fed 0.65S diets had decreased concentrations of butyrate ($P < 0.001$) and valerate ($P < 0.001$) compared with steers fed diets containing 0.42S, but dietary S concentration had no effect ($P = 0.17$) on isobutyrate concentrations. There were interactions ($P \leq 0.04$) between dietary S concentration and grain processing method with respect to 2-m-isovalerate and 3-m-isovalerate, respectively (Table 3.4). Concentration of 2-m-isovalerate was greatest in steers fed SFC-0.65S and least in steers fed DRC-0.42S.

Concentration of 3-m-isovalerate concentration was lesser in cattle fed DRC-0.42S than in cattle fed DRC-0.65S but was not affected by dietary S concentration in SFC based diets.

With respect to total ruminal VFA concentration, there was an interaction (P = 0.006) between dietary S concentration and grain processing method (Figure 3.6). Total ruminal VFA concentrations were less in steers fed SFC-0.65S than in steers SFC-0.42S, but total ruminal VFA concentrations were not affected by dietary S concentration when DRC was fed. These results are most likely driven by the effect of elevated dietary S concentrations on propionate and acetate concentrations as discussed previously. Average ruminal total VFA concentrations were 89.1, 91.4, 91.1, and 77.8 mM, for DRC-0.42S, DRC-0.65S, SFC-0.42S, and SFC-0.65S, respectively.

With respect to ruminal pH, there was no dietary S concentration × grain processing method interaction (P = 0.69; Figure 3.7). Ruminal pH was greater (P = 0.001) in steers fed 0.65S diets than in steers fed 0.42S diets. This may be attributable to two factors: decreased VFA concentrations (P = 0.04) resulting from less feed intake and greater (P < 0.01) ruminal ammonia concentrations when 0.65S was fed.

Grain processing method had no effect on ruminal butyrate concentration, or A:P ratio, but steers fed SFC-based diets tended (P = 0.06) to have greater lactate concentrations than steers fed DRC-based diets. Contrary to our results, increases in ruminal butyrate and the A:P ratio when feeding DRC vs. SFC were reported by Corona et al. (2006) and May et al. (2009). The lack of grain processing effect on these parameters in the current study may be attributable to low inclusion level of grain in experimental diets (Table 3.2).

Steers decreased feed intake in response to chronic exposure to 0.65% S (DM) and had compensatory greater total tract DM digestibility. Decreased feed intake associated with 0.65 S led to increased ruminal ammonia concentrations, particularly in steers fed DRC-based diets, but decreased total VFA concentrations only in steers fed SFC-based diets.

Overall, feeding agricultural by-products that contain elevated S concentrations, such as DDGS, could reduce energetic efficiency by adversely affecting feed intake, producing more undesirable hydrogen sulfide and less propionate, and compromising growth performance of feedlot cattle.

References

AOAC. 1984. Official Methods of Analysis. 14th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 1990. Official Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 1995. Official Methods of Analysis. 16th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Bach, A., S. Calsamiglia, and M. D. Stern. 2005. Nitrogen metabolismin the rumen. J. Dairy Sci. 88(E. Suppl.):9–21.

Barajas, R., and R. A. Zinn. 1998. The feeding value of dry-rolled corn and steam-flaked corn in finishing diets for feedlot steers: Influence of protein supplementation. J. Anim. Sci. 76:1744-1752.

Bird, P. R. 1972. Sulphur metabolism and excretion studies in ruminants X. Sulphide toxicity in sheep. Aust. J. Biol. Sci. 25:1087-1098.

Bird, P. R., and I. D. Hume. 1971. Sulphur metabolism and excretion studies in ruminants. IV. Cystine and sulphate effects upon the flow of sulphur from the rumen and upon sulphur excretion by sheep. Aust. J. Agric. Res. 22:443-452.

Boila, R. J., and L. S. Golfman. 1991. Effects of molybdenum and sulfur on digestion by steers. J. Anim. Sci. 69:1626-1635.

Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. J. Dairy Sci. 63:64-75.

Buckner, C. D., T. L. Mader, G. E. Erickson, S. L. Colgan, K. K. Karges, and M. L. Gibson. 2007. Optimum concentrations of dry distillers grains with solubles for finishing beef steers. Pages 36-38 in Beef Steers Report MP 90. University of Nebraska, Lincoln.

Cooper, R. J., C. T. Milton, T. J. Klopfenstein, and D. J. Jordan. 2002. Effect of corn processing method on degradable intake protein requirement for finishing steers. J. Anim. Sci. 80:242–247.

Corona, L. C., F. N. Owens, and R. A. Zinn. 2006. Impact of corn vitreousness and processing on site and extent of digestion by feedlot steers. J. Anim. Sci. 84:3020-3031.

Cummings, B. A., D. R. Caldwell, D. H. Gould, and D. W. Hamar. 1995a. Identity and interactions of rumen microbes associated with dietary sulfate-induced polioencephalomalacia in cattle. Am. J. Vet. Res. 56:1384-1389.

Cummings, B. A., D. H. Gould, D. R. Caldwell, and D. W. Hamar. 1995b. Ruminal microbial alterations associated with sulfide generation in steers with dietary sulfate-induced polioencephalomalcia. Am. J. Vet. Res. 56:1390-1395.

Dar, S. A., R. Kleerebezem, J. G.Kuenen, and G. Muyzer. 2008. Competition and coexistence of sulfate –reducing bacteria, acetogens and methanogens in a lab-scale anaerobic bioreactor as affected by changing substrate to sulfate ratio. Appl. Microbiol. Biotechnol. 78:1045-1055.

Gochman, N., and J. M. Schimitz. 1972. Application of new peroxide indicator reaction to specific automated determination of glucose with glucose oxidase. Clin. Chem. 18:943-950.

Gould, D. H. 1998. Polioencephalomalacia. J. Anim. Sci. 76:309-314.

Gould, D. H. 2000. Update on sulfur-related polioencephalomalacia. Vet. Clin. North Am. Food Anim. Pract. 16:481- 495.

Herrera-Saldana R., and J. T. Huber. 1989. Influence of varying protein and starch degradabilities on performance of lactating cows. J. Dairy Sci. 72:1477-1483.

Holt, S. M., and R. H. Pritchard. 2004. Composition and nutritive value of corn co-products from dry milling ethanol plants. Pages 1–7 in SD Beef Rep. BEEF 2004-01. South Dakota Agric. Exp. Stn., Brookings.

Hume, I. D. and P. R. Bird. 1970. Synthesis of microbial protein in the rumen IV. The influence of the level and form of sulphur. Aust. J. Agric. Res. 21:315-322.

Kandylis, K. 1984. Toxicology of S in ruminants: Review. J. Dairy Sci. 67:2179-2187.

Kang-Meznarich, J. H. and G. A. Broderick. 1981. Effects of incremental urea supplementation on ruminal ammonia concentration and bacterial protein formation. J. Anim. Sci. 51:422-431.

Klopfenstein, T. J., G. E. Erickson, and V. R. Bremer. 2008. BOARD-INVITED REVIEW: Use of distillers by-products in the beef cattle feeding industry. J. Anim. Sci. 86:1223-1231.

Kung, L., A. O. Hession, and J. P. Bracht. 1998. Inhibition of sulfate reduction to sulfide by 9, 10-anthraquinone in *in vitro* ruminal fermentations. J. Dairy Sci. 81:2251-2256.

May, L. M., M. J. Quinn, C. D. Reinhardt, L. Murray, M. L. Gibson, K. K. Karges, and J. S. Drouillard. 2009. Effects of dry-rolled or steam-flaked corn finishing diets with or without 25% distillers dried grains on ruminal fermentation and apparent total tract digestion. J. Anim. Sci. 87: 3630-3638.

May, L. M, J. C. DeClerck, J. Leibovich, M. J. Quinn, N. DiLorenzo, D. R. Smith, K. E. Hales,

and M. L. Galyean. 2010. Corn or sorghum wet distillers grains with solubles in combination with steam-flaked corn: *In vitro* fermentation and hydrogen sulfide production. J. Anim. Sci. 88: 2425-2432.

Nichols, C. A., K. M. Rolfe, C. D. Buckner, G. E. Erickson, T. J. Klopfenstein. 2011. Effect of source and level of sulfur on rumen metabolism and finishing performance. Beef Cattle Report MP 94. Pages 60-61. University of Nebraska, Lincoln.

NRC. 2000. Nutrient Requirements of Beef Steers. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Qi, K., C. D. Lu, and F. N. Owens. 1993. Sulfate supplementation of growing goats: Effects on performance, acid-base balance, and nutrient digestibilities. J. Anim. Sci. 71:1579-1587.

Rumsey, T. S. 1978. Effects of dietary S addition and Synovex-S ear implants on feedlot steers fed an all-concentrate finishing ration. J. Anim. Sci. 46:463-477.

Russell, J. B. 2002. Page 45 in Rumen Microbiology and Its Role in Ruminant Nutrition. Dep. of Microbiol., Cornell Univ., Ithaca, NY.

Sarturi J. O., G. E. Erickson, T. J. Klopfenstein, J. T. Vasconcelos, K. Rolfe, and M. G. Dib. 2010. Effects of sulfur content of wet or dry distillers grains in beef cattle finishing diets on intake, ruminal pH, and hydrogen sulfide. J. Anim. Sci. 88(E Suppl. 3):557. (Abstr.)

Sindt, J. J., J. S. Drouillard, S. P. Montgomery, and T. B. Farran. 2000. Refractive index: A rapid method for determination of starch availability in grains. Cattlemen's Day SRP 850. Pages 62-63. Kansas Agric. Exp. Stn., Manhattan. Accessed Mar. 17, 2011. http://www.ksre.ksu.edu/library/lvstk2/srp850.pdf.

Smith, D. R., N. DiLorenzo, J. Leibovich, M. L. May, M. J. Quinn, J. W. Homm, and M. L. Galyean. 2010. Effects of sulfur and monensin concentrations on *in vitro* dry matter disappearance, hydrogen sulfide production, and volatile fatty acid concentrations in batch culture ruminal fermentations. J. Anim. Sci. 88:1503–1512.

Spiehs, M. J., M. H. Whitney, and G. C. Shurson. 2002. Nutrient database for distillers dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. J. Anim. Sci. 80:2639–2645.

Theurer, C. B. 1986. Grain processing effects on starch utilization in ruminants. J. Anim. Sci. 63:1649-1663.

Thompson, L. H., M. B. Wise, R. W. Harvey, and E. R. Barrick. 1972. Starea, urea and sulfur in beef steers rations. J. Anim. Sci. 35:474-480.

Undersander, D., R. D. Mertens, and N. Thiex. 1993. Forage Analysis Procedures. Natl. Forage Testing Assoc., Omaha, NE.

Uwituze, S., G. L. Parsons, C. J. Schneider, K. K. Karges, M. L. Gibson, L. C. Hollis, J. J. Higgins, and J. S. Drouillard. 2011. Evaluation of sulfur content of dried distillers grains with solubles in finishing diets based on steam-flaked corn or dry-rolled corn J. Anim Sci. doi:10.2527/jas.2010-3103.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to steer nutrition. J. Dairy Sci. 74:3583-3597.

Whanger, P. D. and G. Matrone. 1966. Effect of dietary sulfur upon the production and absorption of lactate in sheep. Biochim. Biophys. Acta. 124: 273- 279.

Whanger, P. D. and G. Matrone. 1967. Metabolism of lactic, succinic, and acrylic acids by rumen microorganisms from sheep fed sulfur-adequate and sulfur-deficient diets. Biochim. Biophys. Acta. 136: 27- 35.

Widdle, F. 1988. Microbiology and ecology of sulfate reducing bacteria. Page 469 in Biology of anaerobic microorganisms. Zehnder AJB ed. Wiley, New York, NY.

Williams, C. H., D. J. David, and O. Iisamaa. 1962. The determination of chromic oxide in feces samples by atomic absorption spectrophotometry. J. Agri. Sci. 59:381-385.

Zinn, R. A. 1990. Influence of steaming time on site of digestion of flaked corn in steers. J. Anim. Sci. 68:776–781.

Zinn, R. A., E. Alvarez, M. Mendez, M. Montaño, E. Ramirez, and Y. Shen. 1997. Influence of dietary sulfur concentration on growth performance and digestive function in feedlot steers. J. Anim. Sci. 75:1723-1728.

Table 3.1 Composition of corn dried distillers grains with solubles (DDGS) used in experimental diets based on steam-flaked corn or dry-rolled corn fed to ruminally cannulated steers

Item, % DM	Untreated DDGS[1]	Treated DDGS[2]
DM	88.9	88.2
OM	94.7	94.6
CP	29.4	29.0
NDF	27.7	26.7
Starch	5.8	5.6
Ether extract	12.0	12.0
Ca	0.26	0.26
P	0.92	0.88
S	0.97	1.70

[1] Obtained from Poet Nutrition (Sioux Falls, SD).

[2] Ten kilograms of H_2SO_4 (93% concentration) were mixed with 446 kg of DDGS to attain 1.70% S.

Table 3.2 Composition of finishing diets based on steam-flaked corn or dry-rolled corn containing dried distillers grains with solubles (DDGS) with 0.42 (0.42S) or 0.65% S (0.65S) fed to ruminally cannulated steers

	Dry-rolled corn		Steam-flaked corn	
Item	0.42S	0.65S	0.42S	0.65S
Ingredients, % DM				
Steam-flaked corn	-	-	51.1	50.6
Dry-rolled corn	51.3	50.8	-	-
DDGS with high sulfur	-	30.4	-	30.6
DDGS with moderate sulfur	29.9	-	30.1	-
Alfalfa hay	8.5	8.6	8.6	8.6
Cane molasses	6.2	6.2	6.2	6.2
Supplement[1]	4.0	4.0	4.0	4.0
Analyzed composition , %				
DM	87.2	86.6	84.1	83.4
Starch	38.3	38.4	38.8	38.9
CP	15.6	15.4	15.2	15.0
Ether extract	5.8	5.8	5.8	5.8
NDF	12.6	12.2	12.5	12.1
Ca	0.7	0.7	0.7	0.7
P	0.4	0.4	0.4	0.4
K	0.7	0.7	0.7	0.7
S	0.42	0.65	0.42	0.65

[1]Formulated to provide 300 mg/d monensin (Elanco Animal Health, Indianapolis, IN), 90 mg/d tylosin (Elanco Animal Health, Indianapolis, IN), 2200 IU/kg vitamin A, 22 IU vitamin E, 10 mg/kg Cu, 60 mg/kg Zn, 60 mg/kg Mn, 0.5 mg/kg I, 0.25 mg/kg Se, and 0.15 mg/kg Co.

Table 3.3 Nutrient intake and digestion characteristics in ruminally cannulated crossbred steers fed dry-rolled corn or steam-flaked corn diets containing 30% (DM) dried distillers grains with 0.42 (0.42S) or 0.65% S (0.65S)

	Dry-rolled corn		Steam-flaked corn			*P* values		
	0.42S	0.65S	0.42S	0.65S	SEM	Grain processing	S concentration	Grain × S concentration
n	6	5	6	5				
Intake, kg/d								
DM	6.76	5.96	6.89	6.25	0.91	0.59	0.08	0.85
OM	6.37	5.62	6.52	5.91	0.86	0.55	0.08	0.85
Starch	2.59	2.29	2.68	2.43	0.35	0.46	0.08	0.85
NDF	0.91	0.74	0.87	0.74	0.13	0.77	0.04	0.77
CP	1.05	0.92	1.05	0.93	0.14	0.95	0.06	0.85
Ether extract	0.39	0.34	0.40	0.36	0.52	0.59	0.08	0.86
Apparent total tract digestibility, %								
DM	70.1	76.1	76.6	79.9	2.1	0.03	0.04	0.59
OM	73.5	78.5	78.8	81.8	2.1	0.06	0.08	0.61
Starch	90.2	96.4	99.2	99.6	2.1	0.02	0.14	0.19
NDF	15.3	21.3	18.4	27.3	6.1	0.37	0.16	0.75
CP	76.5	77.1	77.6	81.9	2.0	0.13	0.18	0.32
Ether extract	90.6	91.4	91.0	94.6	0.7	0.08	0.03	0.14

Figure 3.1 Ruminal ammonia concentrations in ruminally cannulated crossbred steers fed finishing diets based on steam-flaked corn (SFC, triangles) or dry-rolled corn (DRC, squares) containing dried distiller's grains with solubles with 0.42% S (0.42S, open symbols) or 0.65% S (0.65S, solid symbols).

There were 5, 6, 5, and 6 observations for DRC with 0.65S, DRC with 0.42S, and SFC with 0.65S, and SFC with 0.42S, respectively. There were no 3- or 2- way interactions between time after feeding and grain processing method or S concentration ($P \geq 0.13$). There was an interaction between grain processing method and dietary S concentration ($P < 0.01$), effect of grain processing method ($P < 0.01$), effect of dietary S concentration ($P < 0.01$), and effect of time post-feeding ($P < 0.01$). Error bars represent SE.

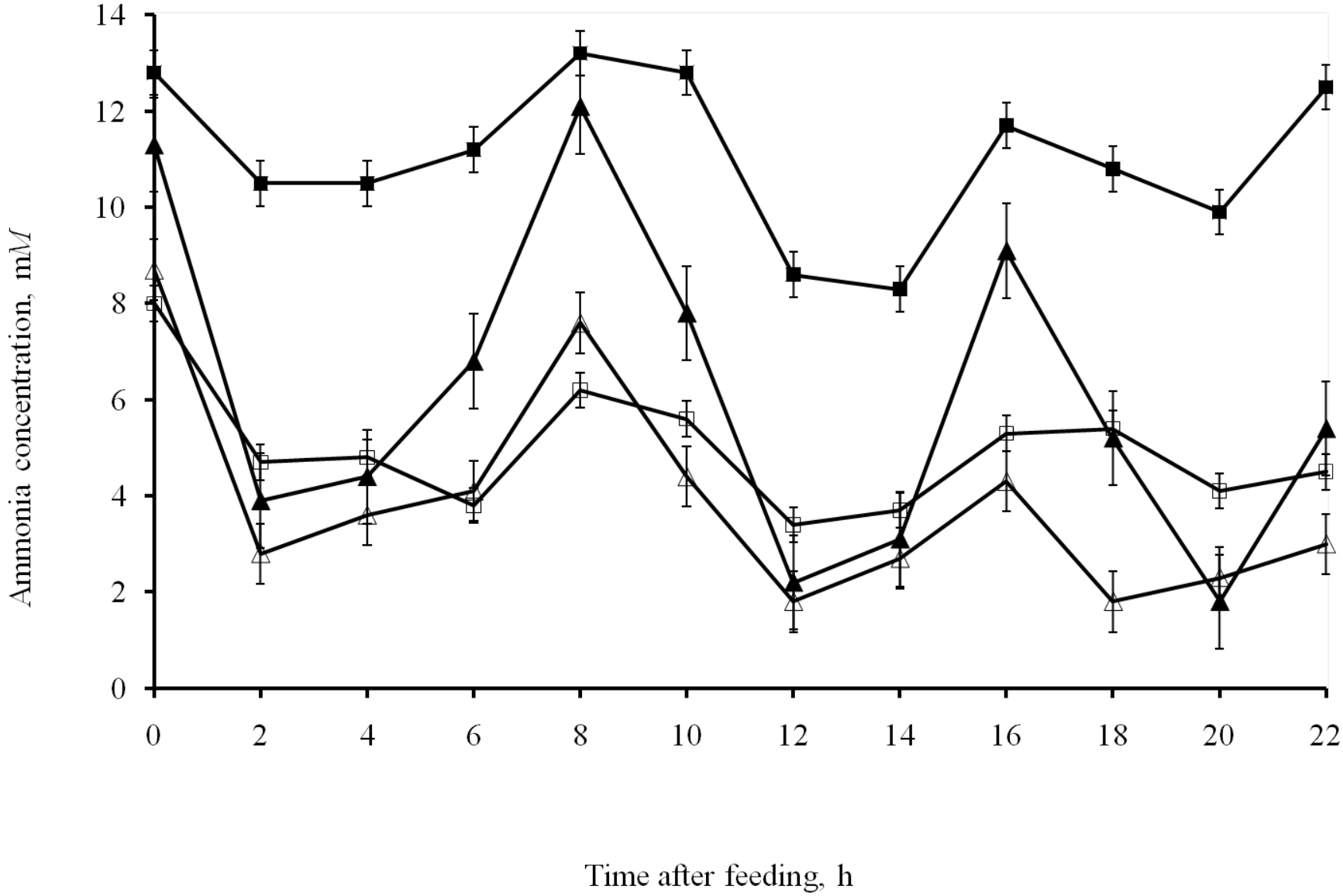

Ammonia concentration, mM
14
12
10
8
6
4
2
0
0
2
4
6
8
10
12
14
16
18
20
22
Time after feeding, h

Figure 3.2 **Ruminal acetate concentrations in ruminally cannulated crossbred steers fed finishing diets based on steam-flaked corn (SFC, triangles) or dry-rolled corn (DRC, squares) containing dried distiller's grains with solubles with 0.42% S (0.42S, open symbols) or 0.65% S (0.65S, solid symbols)**.

There were 5, 6, 5, and 6 observations for DRC with 0.65S, DRC with 0.42S, and SFC with 0.65S, and SFC with 0.42S, respectively. There were no 3- or 2- way interactions between time after feeding and grain processing method or S concentration ($P \geq 0.20$). There was an interaction between grain processing method and dietary S concentration ($P = 0.02$), effect of grain processing method ($P < 0.01$), and effect of time post-feeding ($P < 0.01$). Error bars are SE

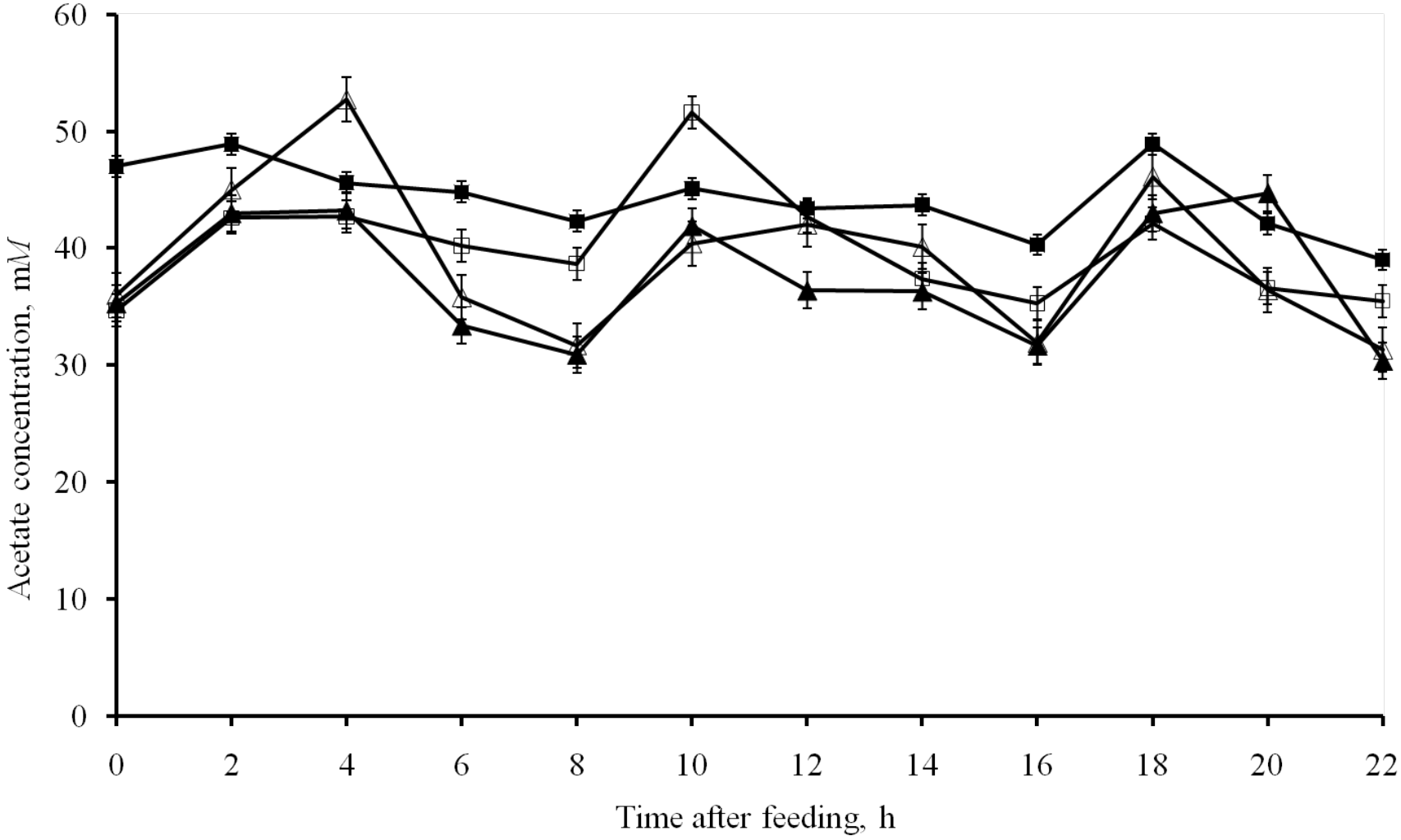

60
50
40
30
20
10
0
Acetate concentration, m*M*
0
2
4
6
8
10
12
14
16
18
20
22
Time after feeding, h

Figure 3.3 Ruminal propionate concentrations in ruminally cannulated crossbred steers fed finishing diets based on steam-flaked corn (SFC, triangles) or dry-rolled corn (DRC, squares) containing dried distiller's grains with solubles with 0.42% S (0.42S, open symbols) or 0.65% S (0.65S, solid symbols).

There were 5, 6, 5, and 6 observations for DRC with 0.65S, DRC with 0.42S, and SFC with 0.65S, and SFC with 0.42S, respectively. There were no 3- or 2- way interactions between time after feeding and grain processing method or S concentration ($P \geq 0.20$). There was an interaction between grain processing method and dietary S concentration ($P < 0.01$), effect of dietary S concentration ($P < 0.01$), and effect of time post-feeding ($P < 0.01$). Error bars are SE

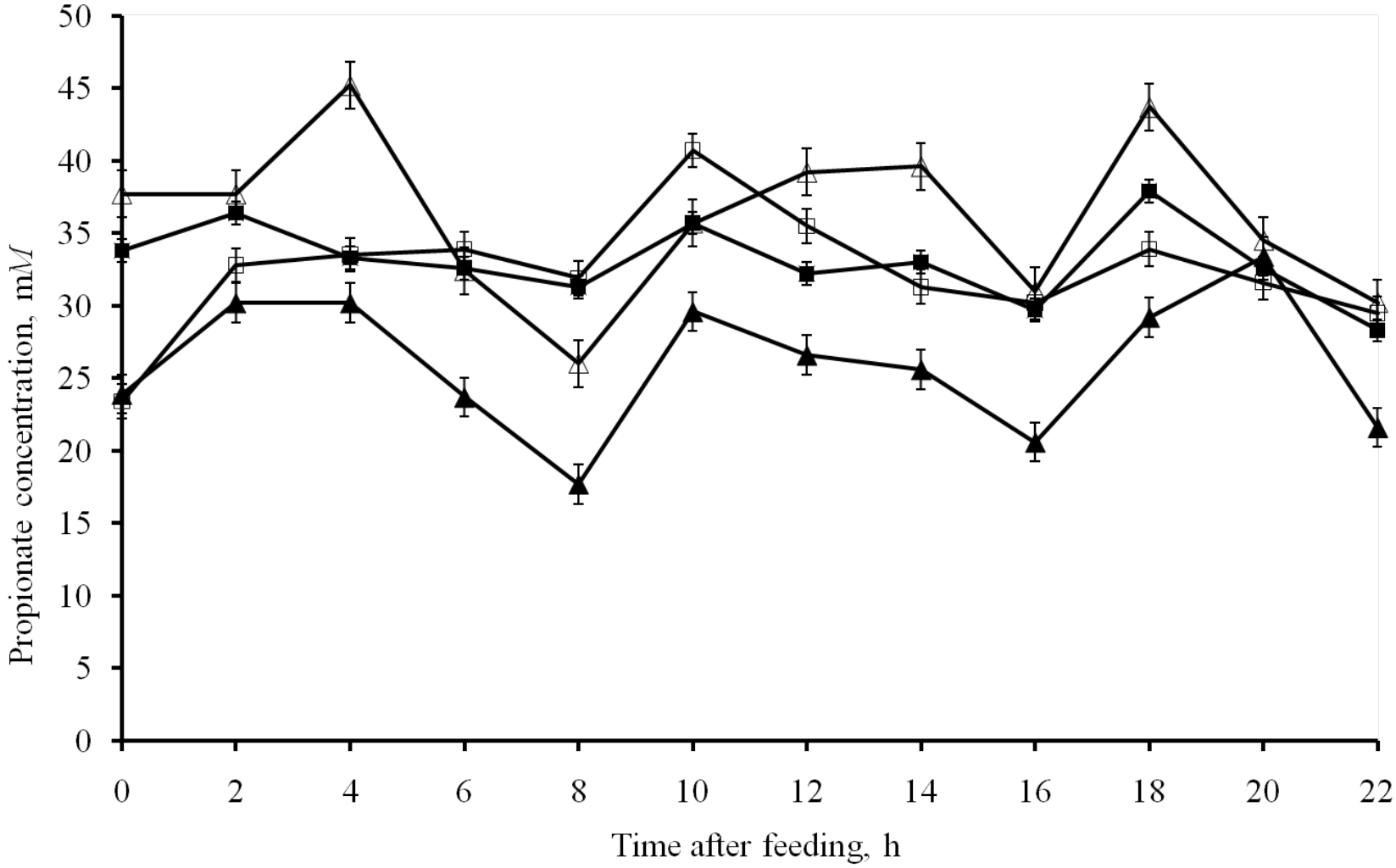

Propionate concentration, mM
50
45
40
35
30
25
20
15
10
5
0
0
2
4
6
8
10
12
14
16
18
20
22
Time after feeding, h

Figure 3.4 Ruminal lactate concentrations in ruminally cannulated crossbred steers fed finishing diets based on steam-flaked corn (SFC, triangles) or dry-rolled corn (DRC, squares) containing dried distiller's grains with solubles with 0.42% S (0.42S, open symbols) or 0.65% S (0.65S, solid symbols).

There were 5, 6, 5, and 6 observations for DRC with 0.65S, DRC with 0.42S, and SFC with 0.65S, and SFC with 0.42S, respectively. There were no 3- or 2- way interactions between time after feeding and grain processing method or S concentration ($P \geq 0.53$), interaction between grain processing method and S concentration ($P = 0.37$) . There were tendencies for grain processing method ($P = 0.06$) S concentration ($P = 0.06$), and time post-feeding ($P = 0.09$). Error bars are SE

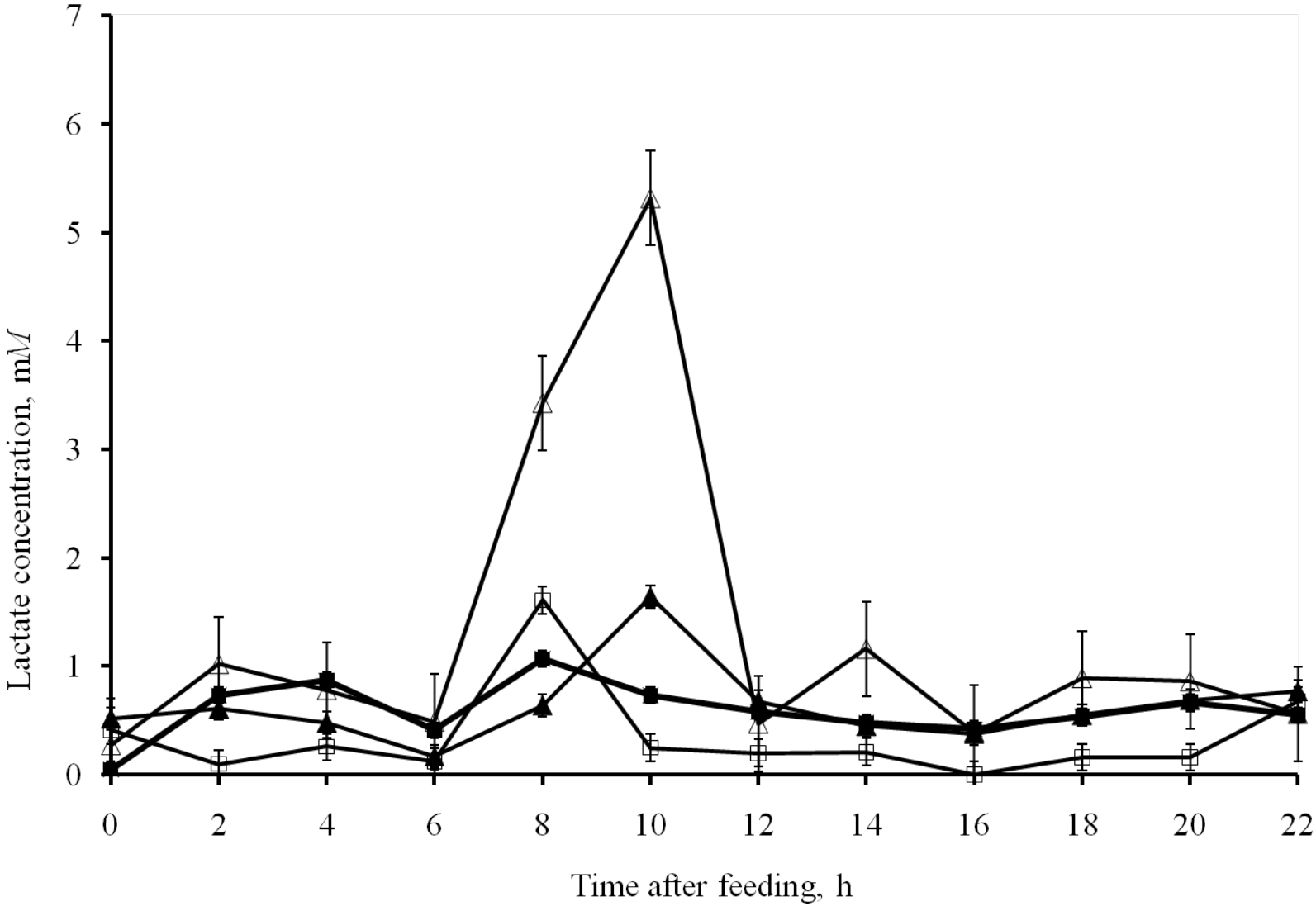

Lactate concentration, mM
0
1
2
3
4
5
6
7
0
2
4
6
8
10
12
14
16
18
20
22
Time after feeding, h

Figure 3.5 Ruminal butyrate concentrations in ruminally cannulated crossbred steers fed finishing diets based on steam-flaked corn (SFC, triangles) or dry-rolled corn (DRC, squares) containing dried distiller's grains with solubles with 0.42% S (0.42S, open symbols) or 0.65% S (0.65S, solid symbols).

There were 5, 6, 5, and 6 observations for DRC with 0.65S, DRC with 0.42S, and SFC with 0.65S, and SFC with 0.42S, respectively. There were no 3- or 2- way interactions between time after feeding and grain processing method or S concentration ($P \geq 0.19$). There was an effect of dietary S concentration ($P < 0.01$) and an effect of time post-feeding ($P = 0.01$). Error bars are SE

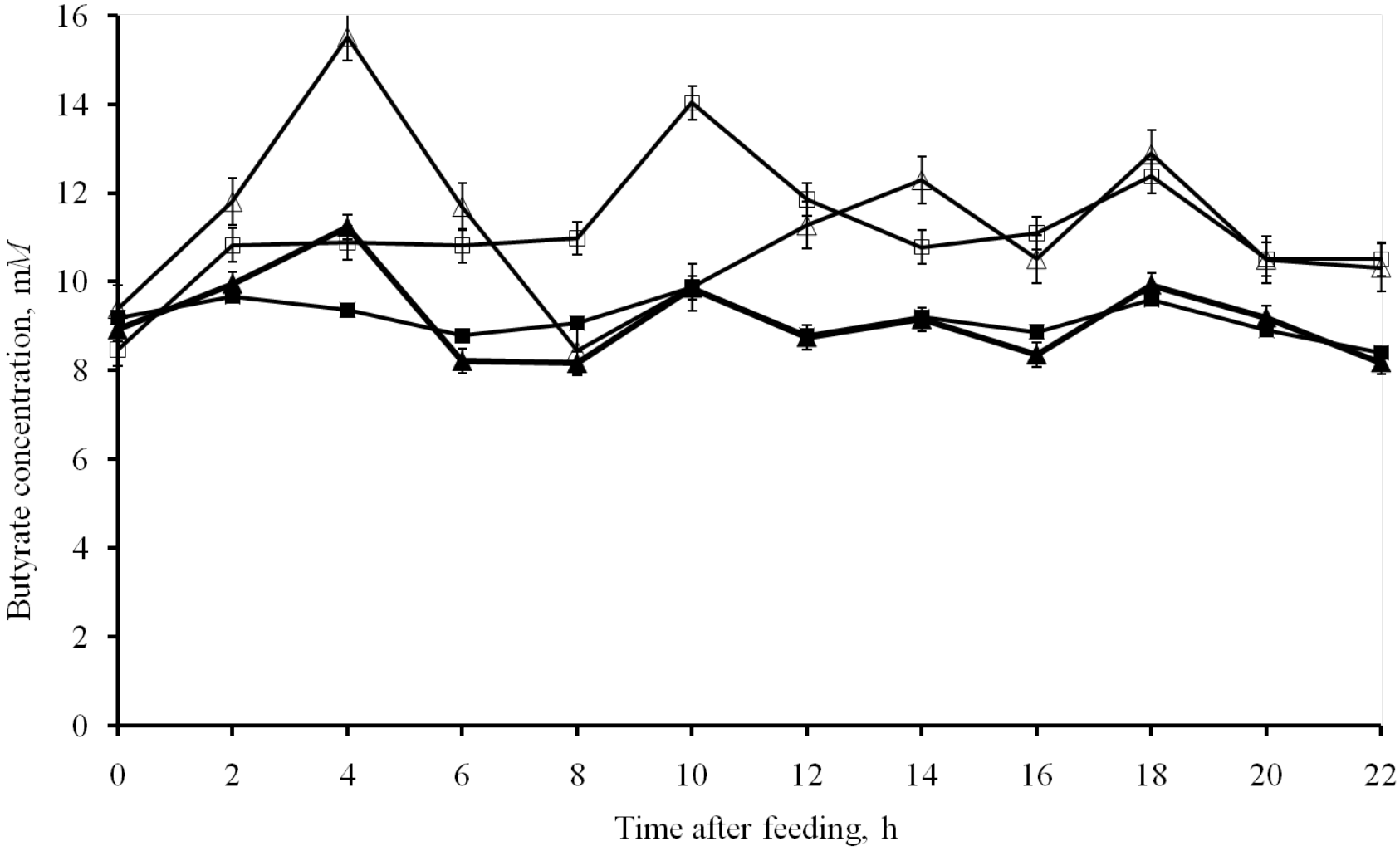

Butyrate concentration, mM
16
14
12
10
8
6
4
2
0
0
2
4
6
8
10
12
14
16
18
20
22
Time after feeding, h

Table 3.4 Ruminal concentrations of minor VFA in ruminally cannulated crossbred steers fed dry-rolled corn or steam-flaked corn diets containing 30% (DM basis) dried distiller's grains with solubles with 0.42 (0.42S) or 0.65% S (0.65S)

	Dry-rolled corn		Steam-flaked corn			*P* values		
Item	0.42S	0.65S	0.42S	0.65S	SEM	Grain processing	S concentration	Grain × S concentration
n	6	5	6	5				
Isobutyrate, m*M*	0.70	0.78	0.68	0.68	0.09	0.06	0.17	0.23
2-*m* Isovalerate, m*M*	0.95	0.52	0.81	1.12	0.17	< 0.01	0.37	<0.001
3-*m* Isovalerate, m*M*	0.66	0.84	0.71	0.72	0.11	0.43	0.01	0.04
Valerate, m*M*	3.39	2.72	3.75	2.62	0.52	0.37	<0.01	0.12

Figure 3.6 Ruminal total VFA concentrations in ruminally cannulated crossbred steers fed finishing diets based on steam-flaked corn (SFC, triangles) or dry-rolled corn (DRC, squares) containing dried distiller's grains with solubles with 0.42% S (0.42S, open symbols) or 0.65% S (0.65S, solid symbols).

There were 5, 6, 5, and 6 observations for DRC with 0.65S, DRC with 0.42S, and SFC with 0.65S, and SFC with 0.42S, respectively. There were no 3- or 2- way interactions between time after feeding and grain processing method or S concentration ($P \geq 0.15$). There was an interaction between grain processing method and dietary S concentration ($P < 0.01$), effect of grain processing method ($P = 0.04$), effect of dietary sulfur concentration ($P = 0.04$), and effect of time post-feeding ($P < 0.01$). Error bars represent SE.

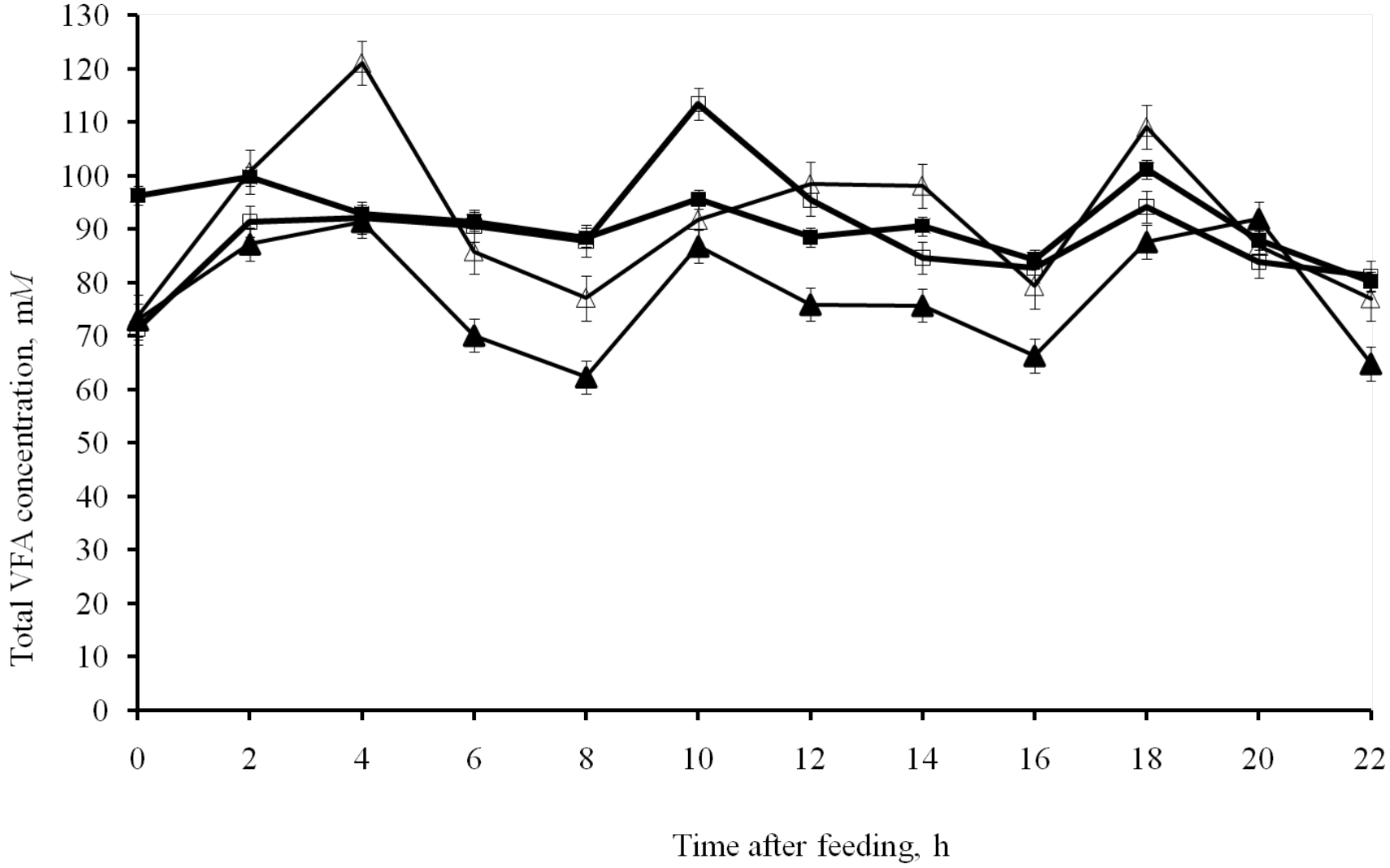
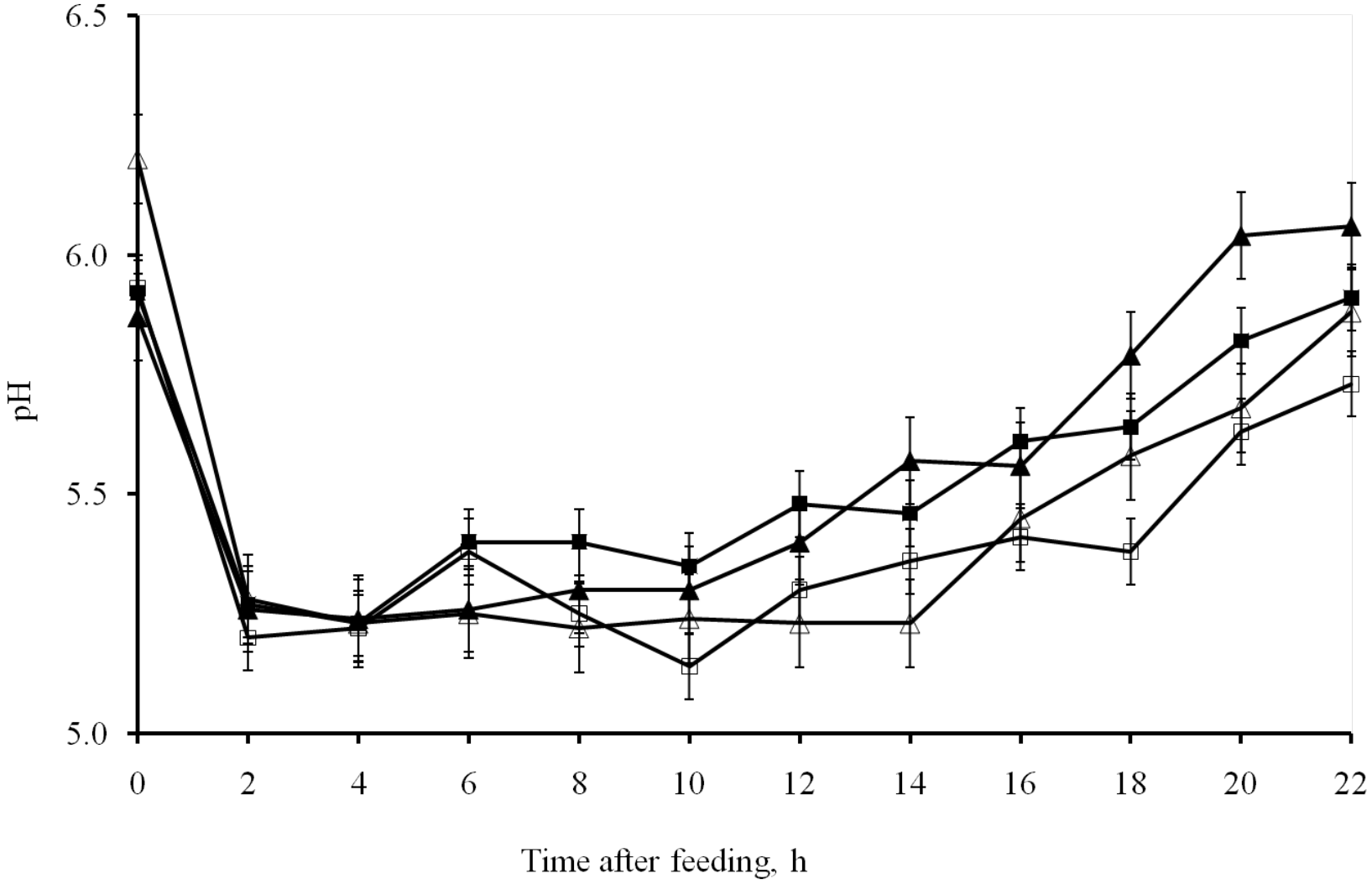

Total VFA concentration, mM
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
22
Time after feeding, h

Figure 3.7 Ruminal pH in ruminally cannulated crossbred steers fed finishing diets based on steam-flaked corn (SFC, triangles) or dry-rolled corn (DRC, squares) containing dried distiller's grains with solubles with 0.42% S (0.42S, open symbols) or 0.65% S (0.65S, solid symbols).

There were 5, 6, 5, and 6 observations for DRC with 0.65S, DRC with 0.42S, and SFC with 0.65S and SFC with 0.42S, respectively. There were no 3- or 2- way interactions between time after feeding and grain processing method or S concentration ($P \geq 0.16$).There were an effect of dietary S concentration ($P < 0.01$) and an effect of time post-feeding ($P < 0.01$). Error bars are SE


6.5
6.0
5.5
5.0
pH
0
2
4
6
8
10
12
14
16
18
20
22
Time after feeding, h

Chapter 4 - Effects of added sulfur on *in vitro* fermentative activity of ruminal microorganisms

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Abstract

We previously reported that elevated S concentrations in finishing diets containing dried distillers grains with solubles (DDGS) decreased DMI and ADG of feedlot cattle, but increased diet digestibility *in vivo*. Two *in vitro* studies were conducted to investigate effects of added S to 2 different substrates on fermentation by mixed ruminal microorganisms. Concentrations of VFA and ammonia, and IVDMD were evaluated in Exp. 1, which was performed as a randomized complete block design with a 2 × 7 factorial treatment arrangement. Factor 1 was substrate (a 94:4.5:1.5 mixture of ground corn, soybean meal, and urea [GC-SBM], or a 69.4:30.6 mixture of ground corn and DDGS [GC-DDGS]). Factor 2 was concentration of added S (0; 0.1; 0.2; 0.3; 0.4; 0.5; or 0.6% of substrate, DM basis) using Na_2SO_4 as the S source. Substrates (0.5 g DM) with varying S concentrations were combined with a 2:1 mixture of McDougall's buffer and strained ruminal fluid from 1 donor steer (fed 40% alfalfa and dry-rolled corn) and incubated in triplicate for 24 h at 39C. The study was repeated for 3 d. Concentrations of ammonia, total VFA, and IVDMD were unaffected by S ($P > 0.05$) or by the S × substrate interaction ($P > 0.05$). Acetate concentration decreased with increasing S concentration in tubes containing GC-DDGS, whereas it increased quadratically in tubes containing GC-SBM plateauing at 0.2% S. Propionate concentration increased quadratically with increasing S concentrations up to 0.5% S. Cultures with GC-DDGS yielded lower concentrations of ammonia, propionate, and butyrate, and had less IVDMD than GC-SBM cultures ($P < 0.05$). *In vitro* gas production and composition were evaluated in Exp. 2, which was conducted as a randomized complete block design with a 2 × 4 factorial treatment arrangement. Substrates used were the same as in Exp. 1, and 0; 0.2; 0.4; or 0.6% S were added as Na_2SO_4. Ruminal fluid was collected from 1 ruminally cannulated steer fed same diet as in Exp. 1. Substrates (1.5 g DM) and buffered ruminal fluid were placed into fermentation flasks equipped with pressure sensitive membranes and radiofrequency transmitters that recorded pressure of fermentative gasses. Cultures were incubated in duplicate for 24 h at 39C, and the trial was repeated for 3 d. *In vitro* gas production and gas composition were not affected by the S × substrate interaction ($P > 0.05$) or by S ($P > 0.05$). Sulfur appears to slightly alter composition of fermentation end products without affecting rate or extent *in vitro* microbial fermentation.

Keys words: Distillers grains, *in vitro* fermentation, sulfur

Introduction

Sulfur is reduced to sulfide in the rumen, and also is used for microbial synthesis of cysteine and methionine (Block et al., 1951; Thomas et al., 1951), thus contributing to increasing microbial protein yield, overall fermentation activity, and diet digestibility (Hume and Bird. 1970). NRC (2000) recommends between 0.14 and 0.40% S of DM for feedlot cattle. Common by-product feedstuffs such as distillers grains with solubles (DGS) can contain high S concentration (Spiehs et al., 2002; Buckner et al., 2011). Excessive dietary S has been associated with decreased feedlot performance (Zinn et al., 1997; Spears et al., 2011) and increased incidence of polioencephalomalacia (PEM, Gould, 1998); hence, researchers are interested in S metabolism in ruminants.

We previously reported that elevated concentrations of dietary S (0.65% S DM basis) in finishing diets containing dried distillers grains with solubles (DDGS) decreased DMI and ADG by feedlot cattle (Uwituze et al., 2011a). Furthermore, feeding 0.65% S yielded lower ruminal concentrations of VFA, but was associated with increased ruminal ammonia concentration and improved total tract diet digestibility compared to feeding 0.42% S (Uwituze et al., 2011b). Smith et al. (2010) reported no effect of 0.2, 0.4, and 0.8% S DM on *in vitro* fermentative activity of micorganisms from steers fed steam-flaked corn-based finishing diets and fermenting steam-flaked corn - based substrate. The objective of this study was to ferment 2 substrates containing different S concentrations *in vitro* to identify threshold concentrations at which dietary S depresses microbial activity of ruminal microorganisms.

Materials and Methods

All described procedures involving live animals were approved by the Kansas State University Institutional Animal Care and Use Committee protocol no. 2615.

Experimental Design

Effects of added S on ammonia concentration, VFA profiles, and IVDMD were evaluated in Exp. 1. The study was conducted as a randomized complete block design using a factorial arrangement of 2 substrate types and 7 concentrations of added S. Substrates consisted of a 94:4.5:1.5 mixture of ground corn (GC), soybean meal (SBM), and urea (GC-SBM), or a

69.4:30.6 mixture of GC and DDGS (GC-DDGS). Concentrations of added S were 0; 0.1; 0.2; 0.3; 0.4; 0.5; or 0.6% of substrate on a DM basis.

In vitro gas production and composition of head space gas were evaluated in Exp. 2, which was conducted as complete block design with a 2 × 4 arrangement. Substrates were the same as in Exp. 1, and 0; 0.2; 0.4; or 0.6% S was added (DM basis). Sodium sulfate was used as the S source for both experiments. Inocula for Exp. 1 and 2 consisted of a 2:1 mixture of McDougall's buffer (McDougall, 1948) and clarified ruminal fluid. Both experiments were repeated on 3 separate days. Day was used as the blocking factor.

Ruminal digesta used for both experiments was obtained from a single ruminally cannulated crossbred Angus steer (approximately 650 kg BW) fed a diet consisting of 40% roughage and 60% concentrate (Table 4.1). The steer was housed in a 1.5 × 3.5 m pen with a slatted-floor, individual feed bunk, and a water fountain. Fresh feed was provided once daily *ad libitum* at 0800 h and drinking water was available *ad libitum*. The animal was adapted to the diet for 21 d before collection of ruminal fluid samples and remained on this diet until the end of the experiments. Samples of ruminal fluid were collected in the morning before feeding.

Substrate Preparation and Composition

Samples of GC, SBM, and DDGS were analyzed for 105C DM (Undersander et al., 1993), while another set of ingredient samples were dried at 55C for 48 h and ground through a 1-mm screen using a Wiley mill (Arthur H. Thomas Co., Philadelphia PA) before blending them to make GC-SBM and GC-DDGS proportions described previously. Individual ingredients were analyzed for S content following official method 923.01 (AOAC, 1990) and for N content using a N analyzer (FP–2000; LECO Corp., St Joseph, MI) according to AOAC (1995) official method 990.03. Basal S concentrations were 0.18 and 0.28% S (DM) for GC-SBM and GC-DDGS, respectively, and both substrates contained 14.4% CP on DM basis. Diet ingredients were analyzed for DM, NDF, CP, and S as previously described by Uwituze et al. (2011).

Exp. 1: IVDMD and in Vitro Concentrations of Ammonia and VFA

To determine effects of S content on IVDMD and concentrations of ammonia and VFA, substrates (0.5 g DM) were weighed into 50-mL culture tubes. Three culture tubes were used per substrate × S combination, and three blank tubes (containing buffer and ruminal fluid without substrate) were included for each day of the experiment.

Whole ruminal contents were obtained via the ruminal cannula and placed into a thermos pre-heated to 39C. Ruminal contents were subsequently strained through 8 layers of cheesecloth, placed into a large separatory funnel, sparged with CO_2, and placed into a 39C walk-in incubator for 30 to 40 min to allow for stratification of the mat, fluid, and protozoal fractions. The protozoa-rich fraction was voided from the funnel, and the clarified liquid layer was mixed 1:2 with McDougall's buffer. An aliquot of 30 mL of the mixture was added to culture tubes containing substrates and blanks. A stock solution containing 1% (wt/vol) Na_2SO_4 was prepared, and 0.05, 0.10, 0.15, 0.20, 0.25, and 0.30 mL of the stock solution was added to culture tubes containing 30 mL of inoculum to achieve final S concentration of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6% S, respectively. Tubes were then bubbled with CO_2, capped with gas-release stoppers, and placed in a reciprocal oscillating (30 rpm) water bath (Precision Model 25, Thermo Electron Corp., Marietta, OH) at 39C for 24 h. The experiment was repeated on 3 separate d.

After 24 h of fermentation, tubes were chilled in an ice bath and centrifuged at 30,000 × g for 20 min at 4C. A 4-mL aliquot of supernatant was collected, mixed with 1 mL of 25% (wt/vol) metaphosphoric acid, and subsequently frozen at -20C for later analyses of VFA and ammonia concentrations. Pellets of residue were dried at 100C overnight, placed into a desiccator to cool, and weighed to determine IVDMD. *In vitro* dry matter disappearance was calculated for each tube as the difference between the weight of dry sample and the weight of blank-corrected dry residue divided by the weight of dry sample.

Acidified ruminal fluid samples were later thawed to room temperature and centrifuged at 30,000 × g for 20 min, and 1 mL of the supernatant fluid was used to determine concentrations of acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate using a gas chromatograph (5890A; Hewlett-Packard, Palo Alto, CA; 2-m × 4-mm column; GP 10% SP- 1200/1% H_3PO_4 column packing, Supelco Bellefonte, PA) with He as the carrier gas, a flow rate of 60 mL/min, and a column temperature of 130C. Total VFA concentration was computed as the sum of individual VFA concentrations. A portion (1 mL) of the supernatant was analyzed for ruminal ammonia concentration using an Autoanalyzer III (SEAL Analytical, Mequon, WI) following procedures described by Broderick and Kang (1980).

Exp. 2: In Vitro Gas Production and Composition

Ruminal digesta were collected and processed as described in Exp.1. Substrates were the same as those used in Exp. 1. Substrates were weighed (1.5 g DM) into 250-mL flasks equipped with septa (Ankom Technology Corp., Macedon, NY) that allow collection of gas samples from the head-space using a needle. An inoculum consisting of 50 mL of clarified ruminal fluid and 100 mL of McDougall's buffer was added to each flask. After addition of inoculum, 0, 0.3, 0.6, or 0.9 mL of 1% (wt/vol) solution of Na_2SO_4 was added to flasks to obtain 0.2, 0.4, and 0.6% added S concentration, respectively. Two blank flasks containing inoculum without substrate were included. Flasks were subsequently flushed with CO_2 and sealed with gas pressure monitor modules (Ankom Technology Corp., Macedon, NY). Each module was equipped with a pressure-sensitive membrane and a radiofrequency transmitter that recorded pressure generated by fermentative gasses using a base coordinator unit (Gas Pressure Monitor System, Ankom Technology Corp.) connected to a computer. Sealed flasks were placed in a reciprocal shaking water bath (Precision Model 25, Thermo Electron Corp., Marietta, OH) set at 39C, and allowed to ferment under constant agitation (30 rpm) for 24 h. Cumulative gas pressures and total volume of gasses produced were recorded in a spreadsheet.

After a 24-h fermentation period, gas pressures were converted into moles of gas (n) using the following 'ideal' gas law (Ankom Technology Corp., 2011).

$$n = p\,(V/RT)$$

In this formula, n is gas produced (mol), p is pressure (kPa), V is head-space volume in the flasks (L), T is temperature (K), and R is gas constant (8.314472 $L.kPa.K^{-1}.mol^{-1}$). Moles of gas were subsequently converted into volume of gas (mL) using Avogadro's law (Ankom Technology Corp., 2011).

$$\text{Gas produced (mL)} = n \times 25.6 \times 1000$$

Where 25.6 is the volume occupied by 1 mole of gas at 39C.

After 8, 16, and 24 h of incubation, 60 mL samples of fermentative gasses were aspirated from the head-space of flasks by penetrating the septum, with a 25-gauge, 22.2 mm long needle attached to a 60-mL syringe. Aspirated gas samples were immediately injected through the gray butyl stopper capping a vacuumed 30-mL serum bottle, and sealed with aluminum crimps.

To determine gas composition, 10 mL of gas were aspirated from serum bottles into a gas-tight syringe (10 MDF-LL-GT; SGE, Austin, TX) and manually injected into a gas chromatograph (SRI Instruments, Torrance, CA) via a gas sampling valve with a 0.5-mL sample loop. Concentrations of H_2S, CO_2, and CH_4 were determined with a flame photometric detector, a thermal-conductivity detector, and a flame ionization detector, respectively. Separation was performed using a 3 mm × 0.9 m Haye Sep D packed Teflon column (SRI Instruments, Torrance, CA) with helium gas as the carrier. Pressure was maintained at 69 KPa, and oven temperature was maintained at 40C.

Statistical Analyses

Concentrations of VFA and ammonia, and percent IVDMD were analyzed using the Mixed procedure of SAS (version 9.2; SAS Inst., Cary, NC) with fixed effects of substrate, S, and the substrate × S interaction. The random effect was day. Linear, quadratic, cubic, and quartic effects of S concentration were protected by a significant ($P \leq 0.05$) *F*-test. Data for gas production and gas composition were analyzed as repeated measures using the Mixed procedure of SAS. The model statement included substrate, S, fermentation time, substrate × S, fermentation time × substrate, fermentation time × S, and fermentation time × substrate × S interactions. Random effects consisted of day and flask nested within day × substrate × S. *F*-test protected ($P \leq 0.05$) linear, quadratic, and cubic effects of S concentration were analyzed for data pertaining to gas production and composition. Mean separations were *F*-test protected for both experiments and differences were declared significant at $P \leq 0.05$.

Results and Discussion

Exp. 1: IVDMD and in Vitro Concentrations of Ammonia and VFAs

Treatment effects on IVDMD, VFA profiles, and ammonia concentration are summarized in Table 4.2. No interactions occurred between S concentration and substrate type with respect to IVDMD ($P = 0.86$) or concentrations of ammonia ($P = 0.10$), total VFA ($P = 0.08$), or butyrate ($P = 0.32$). These measures also were unaffected by S concentration ($P > 0.20$). There was a substrate × S interaction ($P = 0.02$) with respect to acetate concentration. Acetate concentration decreased with increasing S concentration in tubes containing GC-DDGS, whereas 0.6% S

yielded the greatest acetate concentration, followed by 0.2% S in tubes containing GC-SBM. The control group without added S yielded the lowest acetate concentration when GC-SBM was fermented. There were quadratic ($P = 0.05$) and cubic ($P = 0.05$) effects of S with respect to propionate concentration. There was a decline in propionate concentration at 0.1% S, followed by a slight increase from 0.2 to 0.5% S. Propionate concentration was least in tubes containing 0.6% S. A substrate × S concentration occurred for acetate:propionate (AP) ratio ($P = 0.03$). Increasing S concentration decreased AP ratio when GC-DDGS was fermented, whereas in tubes containing GC-SBM the greatest AP ratio was observed in tubes containing 0.6 % S, followed by 0.2% S. The group without added S had the lowest AP ratio when GC-SBM was used as substrate.

Whanger and Matrone (1967) reported increased propionate concentrations with increasing dietary S concentration, which is in agreement with findings of the current study. In studies by Zinn et al. (1997) and Thompson et al. (1972), increasing dietary S content from 0.15 to 0.25% in SFC-based diets and from 0.12 to 0.37% in ground corn-based diets, respectively, was associated with greater propionate concentrations and lower acetate concentrations *in vivo*. According to Whanger and Matrone (1967), increased propionate concentration with increasing dietary S may be attributable to lactate metabolism through the acrylate pathway. Dar et al. (2008) reported different bacterial profiles as a result of various lactate to sulfate molar ratios. Lactate concentration was not measured in the current study, but we previously reported a decrease in ruminal lactate concentration in steers fed diets containing 0.65% S compared with steers fed diets containing 0.42% S (Uwituze et al., 2011b).

Interactions between substrate and S content occurred for concentrations of isobutyrate ($P = 0.04$) and isovalerate ($P = 0.01$). Isobutyrate concentration decreased in tubes containing GC-DDGS with increasing S concentration ($P = 0.01$), but was not affected by S concentration ($P > 0.05$) when GC-SBM was fermented. Increasing S concentration from 0.1 to 0.4% S decreased isovalerate concentration when GC-DDGS was used as a substrate, whereas the greatest isovalerate concentration was obtained in tubes containing 0.6% S followed by 0.2% S when GC-SBM was fermented. There was a quartic ($P < 0.01$) effect of S concentration on valerate concentration. Low peak concentrations of valerate were observed in tubes containing 0.1 and 0.6% S, whereas high concentrations were obtained in tubes containing 0.3 and 0.5% S.

Lack of dietary S effect on *in vitro* VFA concentrations and IVDMD has been reported with 0.2, 0.4, and 0.8% S (Smith et al., 2010), or 0.17 and 0.42% S (Quinn et al., 2009) in steam-flaked corn (SFC)-based substrates (DM basis). Likewise, Kung et al. (2000) reported no effects of dietary S concentration (0.29 vs 1.09 % S DM) on concentrations of VFA when a substrate consisting of a ground, early market lamb pellet was fermented *in vitro*. Differences between previous reports and the present study for VFA profiles could be attributable to ruminal fluid source. Ruminal fluid in our study was collected from a steer fed a diet consisting of 60% concentrate and 40% roughage (Table 1), whereas Smith et al. (2010) and Quinn et al. (2009) obtained ruminal fluid from animals fed a high-concentrate diet. In the study by Kung et al. (2000), ruminal fluid was collected from steers fed a commercial concentrate diet with a mixture of alfalfa hay and corn silage. Cattle fed high-roughage diets have greater S tolerance than cattle fed high-concentrate diets (NRC, 2005), and this could be partly due to differences in their ruminal pH. According to Kandylis (1984), ruminal sulfate reducing bacteria thrive at approximately pH 6.5, which is found in cattle fed high-roughage diets, but usually not in cattle fed high-concentrate diets.

The present study was designed to allow direct comparison between 2 substrates with different S concentrations. Our intent was to identify threshold concentrations at which dietary S depresses microbial activity under different situations. In spite of treatment effects on individual VFA concentrations, total VFA concentration and IVDMD were unaffected by S concentrations with either substrate. These findings suggest that dietary S concentration may slightly shift microbial population composition and fermentative activity without adversely affecting overall microbial fermentative activity. Cummings et al. (1995a) noted no changes in relative counts of sulfate-reducing bacteria, but they reported reduction in microbial diversity in ruminal fluid from steers fed diets with added sulfate compared to steers fed diets without added sulfate. According to Cummings et al. (1995b), sulfate-reducing bacteria adapt to increased ruminal sulfate concentration between 8 and 12 d of exposure.

Thus, it is conceivable that S effect on ruminal microorganisms is less likely to cause noticeable changes previously observed in cattle performance and *in vivo* diet digestibility associated with high S intake (Uwituze et al., 2011a,b). Decreased cattle performance despite improved diet digestibility observed in our previous work when 0.65% S was fed (Uwituze et al., 2011a,b), was most likely attributable to host factors such as decreased feed intake. Numerous

studies have reported decreased DMI by feedlot cattle fed diets containing dietary S greater than 0.2% DM, whether as added S (Zinn et al., 1997; Spears et al., 2011) or as a result of feeding elevated levels of DGS (Sarturi et al., 2010; Nichols et al., 2011).

Microorganisms fed GC-DDGS substrate produced greater concentrations of valerate ($P < 0.01$) compared to microorganisms fed GC-SBM, but produced smaller concentrations of ammonia, total VFA, propionate, and butyrate and, had poorer IVDMD compared to cultures with GC-SBM ($P < 0.01$, Table 4.2). Differences between substrate types may be attributable to differences in the content of NDF and ruminally degradable protein of individual ingredients (NRC, 2000). The GC-SBM substrate provided more degradable protein but less NDF compared to GC-DDGS substrate. Hence, improved diet digestibility was observed in cultures incubated with GC-SBM substrate compared to cultures incubated with GC-DDGS substrate (May et al. 2009).

Exp. 2: In Vitro Gas Production and Composition

No fermentation time × substrate × S ($P = 0.75$) or fermentation time × S ($P = 0.09$; Figure 4.1) interactions occurred with respect to total gas production, but there was an interaction between substrate and fermentation time ($P < 0.01$). Substrates yielded similar ($P > 0.05$) total gas production from 0 to 12 h, but cultures with GC-SBM had greater ($P < 0.05$) total gas production than cultures with GC-DDGS from 12 through 24 h of incubation. There were no substrate × S interaction ($P = 0.63$) or S effects ($P = 0 .35$) for total gas production (Figure 4.2).

Gas composition over time is summarized in Table 4.3. No 3- or 2- way interactions occurred with respect to CH_4 concentration ($P \geq 0.14$). There were no effects of S concentration ($P = 0.99$) or substrate type ($P = 0.12$), but CH_4 as a proportion of produced gas increased by 2.1% from 8 to 24 h after fermentation ($P < 0.01$).

No H_2S was detected at 8 or 16 h, but it was detected after a 24-h fermentation period; therefore, only data obtained after 24 h incubation are discussed herein. No substrate × S interaction ($P = 0.40$), or effects of substrate ($P = 0.70$) or S concentration ($P = 0.80$), were observed for H_2S concentration.

Smith et al. (2010) reported that increasing S content of SFC-based substrate at 0.2, 0.4, and 0.8% had no effect on *in vitro* total gas production, but S concentration increased *in vitro* H_2S concentration linearly, which is contrary to findings of the current study. In a study by

Quinn et al. (2009), *in vitro* H_2S concentration increased with increasing S concentration from 0.17 to 0.42% of SFC-based substrate DM. *In vitro* total gas production also tended to increase with increasing S concentration. Lack of S effect on H_2S production after 8, 16, and 24 h of fermentation may suggest adaptation by sulfate reducing bacteria. Cummings et al. (1995b) reported sulfate reducing bacteria *in vivo* require between 8 and 12 d adaptation to elevated dietary S before they are able to produce substantial amounts of H_2S.

Some studies have evaluated effects of S from DGS without added S. May et al. (2011) reported no interaction between 15 or 30% (DM) WDGS and 7.5, 10, or 12.5% (DM) alfalfa in SFC-based substrates with respect to *in vitro* gas production or composition. Dietary S concentrations were between 0.18 and 0.31% S. Control substrates without DGS had greater total gas production and lesser H_2S concentration than substrates containing WDGS. DeClerk (2009) evaluated effects of feeding 15 or 30% (DM) corn or sorghum WDGS in SFC-based substrates on *in vitro* gas production and H_2S concentration. Concentration of H_2S increased linearly with increasing levels of DGS regardless of DGS type, but rates of gas production were lower for substrates containing DGS than for the control without DGS. Leibovich et al. (2009) evaluated effects of 15% sorghum DGS on *in vitro* gas production and H_2S concentrations in substrates based on SFC or DRC. Including sorghum DGS decreased gas production, but *in vitro* H_2S production was not affected by sorghum DGS level. In the Declerk (2009) study, S contents for substrates were 0.20 or 0.26 for corn WDGS; and 0.19 or 0.31% S for sorghum WDGS for diets containing 15 or 30% WDGS (DM), respectively. Substrates utilized by Leibovich et al (2009) had S concentrations similar to those of Declerk (2009; 0.23 and 0.21% for 0 and 15% sorghum DGS, respectively). In the current study, total S concentrations ranged from 0.18 to 0.78 and from 0.28 to 0.88% (DM) for GC-SBM and GC-DDGS, respectively.

Differences for gas production observed among studies by Declerk (2009), Leibovich et al. (2009), and May et al. (2011), and those from the present study, Smith et al. (2010), and Quinn et al. (2009) may suggest a confounding effect of S with other nutrients, such as CP and NDF, when comparing substrates with or without DGS vs. substrates with or without added S.

In summary, dietary S altered composition of ruminal microbial fermentation end products only slightly without adversely effecting overall *in vitro* microbial fermentation, irrespective of substrate type. Previously observed deleterious effects of elevated dietary S on

cattle growth performance and carcass characteristics are less likely associated with negative S effect on ruminal microorganisms compared to decreased DMI.

References

Ankom Technology Corporation. 2011. Conversion of pressure to gas production. Page 44 in Ankom RF gas production system operator's manual. Rev E 3/14/11. Ankom Technology Corp. Macedon, NY.

AOAC. 1990. Official Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 1995. Official Methods of Analysis. 16th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Block, R. J., J. A. Stekol, and J. K. Loosli. 1951. Synthesis of S amino acids from inorganic sulfates by ruminants II. Synthesis of cystine and methionine from sodium sulfate by the goat and by the microorganisms of the rumen of the ewe. Arch. Biochem. Biophys. 33:353–363.

Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. J. Dairy Sci. 63:64-75.

Buckner, C.D., M. F. Wilken, J. R. Benton, PAS, S. J. Vanness, V. R. Bremer, T. J. Klopfenstein, P. J. Konoff, and G. E. Erickson. 2011. Nutrient variability for distillers grains plus solubles and dry matter determination of ethanol by-products. Prof. Anim. Sci. 27:57-64.

Cummings, B. A., D. R. Caldwell, D. H. Gould, and D. W. Hamar. 1995a. Identity and interactions of rumen microbes associated with dietary sulfate-induced polioencephalomalacia in cattle. Am. J. Vet. Res. 56:1384-1389.

Cummings, B. A., D. H. Gould, D. R. Caldwell, and D. W. Hamar. 1995b. Ruminal microbial alterations associated with sulfide generation in steers with dietary sulfate-induced polioencephalomalacia. Am. J. Vet. Res. 56:1390-1395.

Dar, S. A., R. Kleerebezem, J. G.Kuenen, and G. Muyzer. 2008. Competition and coexistence of sulfate-reducing bacteria, acetogens and methanogens in a lab-scale anaerobic bioreactor as affected by changing substrate to sulfate ratio. Appl. Microbiol. Biotechnol. 78:1045-1055.

DeClerck, J. C. 2009. Effects of wet corn and sorghum distillers grain on performance carcass characteristics of finishing beef cattle and on in vitro fermentation of steam-flaked corn based substrates. M.S. Thesis. Texas Tech Univ., Lubbock.

Gould, D. H. 1998. Polioencephalomalacia. J. Anim. Sci. 76:309-314.

Hume, I. D. and P. R. Bird. 1970. Synthesis of microbial protein in the rumen IV. The influence of the level and form of sulphur. Aust. J. Agric. Res. 21:315-322.

Kandylis, K. 1984. The role of sulfur in ruminant nutrition. A Review. Livest. Prod. Sci. 11:611-624.

Kung, L., J. P. Bracht, and J. Y. Tavares. 2000. Effects of various compounds on *in vitro* ruminal fermentation and production of sulfide. Anim. Feed Sci. Technol. 84:69–81.

Leibovich, J., J. T. Vasconcelos, and M. L. Galyean. 2009. Effects of corn processing method in diets containing sorghum wet distillers grains plus solubles on performance and carcass characteristics of finishing beef cattle an on in vitro fermentation of diets. J. Anim. Sci. 87:2124-2132.

May, L. M., M. J. Quinn, C. D. Reinhardt, L. Murray, M. L. Gibson, K. K. Karges, and J. S. Drouillard. 2009. Effects of dry-rolled or steam-flaked corn finishing diets with or without 25% distillers dried grains on ruminal fermentation and apparent total tract digestion. J. Anim. Sci. 87: 3630-3638.

May, M. L., M. J. Quinn, N. DiLorenzo, D. R. Smith and M. L. Galyean. 2011. Effects of roughage concentration in steam-flaked corn-based diets containing wet distillers grains with solubles on feedlot cattle performance, carcass characteristics, and in vitro fermentation. J. Anim. Sci. 89:549–559.

McDougall, E. I. 1948. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. Biochem. J. 43:99–109.

Nichols, C. A., K. M. Rolfe, C. D. Buckner, G. E. Erickson, T. J. Klopfenstein. 2011. Effect of source and level of sulfur on rumen metabolism and finishing performance. Beef Cattle Report MP 94. Pages 60-61. University of Nebraska, Lincoln.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th ed. National Academy Press, Washington, D. C.

NRC. 2005. Mineral Tolerance of Animals. 2nd ed. Natl. Acad. Press, Washington, DC.

Quinn, M. J., M. L. May, K. E. Hales, N. DiLorenzo, J. Leibovich, D. R. Smith, and M. L. Galyean. 2009. Effects of ionophores and antibiotics on *in vitro* hydrogen sulfide production, dry matter disappearance, and total gas production in cultures with a steam-flaked corn-based substrate with or without added sulfur. J. Anim. Sci. 87:1705–1713.

Sarturi J. O., G. E. Erickson, T. J. Klopfenstein, J. T. Vasconcelos, K. Rolfe, and M. G. Dib. 2010. Effects of sulfur content of wet or dry distillers grains in beef cattle finishing diets on intake, ruminal pH, and hydrogen sulfide. J. Anim. Sci. 88(E Suppl. 3):557. (Abstr.)

Smith, D. R., N. DiLorenzo, J. Leibovich, M. L. May, M. J. Quinn, J. W. Homm, and M. L. Galyean. 2010. Effects of S and monensin concentrations on *in vitro* dry matter disappearance, hydrogen sulfide production, and volatile fatty acid concentrations in batch culture ruminal fermentations. J. Anim. Sci. 88:1503–1512.

Spears, J. W., K. E. Lloyd, and R. S. Fry. 2011. Tolerance of cattle to high dietary S and effect of dietary cation-anion balance. J. Anim Sci. doi:10.2527/jas.2010-3265.

Spiehs, M. J., M. H. Whitney, and G. C. Shurson. 2002. Nutrient database for distillers dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. J. Anim. Sci. 80:2639–2645.

Thomas, W. E., J. K. Loosli, H. H. Williams, and L. A. Maynard. 1951. The utilization of inorganic sulfates and urea nitrogen by lambs. J. Nutr. 43:515–523.

Thompson, L. H., M. B. Wise, R. W. Harvey, and E. R. Barrick. 1972. Starea, urea and sulfur in beef steers rations. J. Anim. Sci. 35:474-480.

Undersander, D., R. D. Mertens, and N. Thiex. 1993. Forage Analysis Procedures. Natl. Forage Testing Assoc., Omaha, NE.

Uwituze, S., G. L. Parsons, C. J. Schneider, K. K. Karges, M. L. Gibson, L. C. Hollis, J. J. Higgins, and J. S. Drouillard. 2011. Evaluation of S content of dried distillers grains with solubles in finishing diets based on steam-flaked corn or dry-rolled corn J. Anim Sci. doi:10.2527/jas.2010-3103.

Uwituze, S., G. L. Parsons, K. K. Karges, M. L.Gibson, L. C. Hollis, J. J. Higgins and J. S. Drouillard. 2011. Effects of distillers grains with high sulfur concentration on ruminal fermentation and digestibility of finishing diets. J. Anim. Sci. doi:10.2527/jas.2010-3401.

Whanger, P. D. and G. Matrone. 1967. Metabolism of lactic, succinic, and acrylic acids by rumen microorganisms from sheep fed sulfur-adequate and sulfur-deficient diets. Biochim. Biophys. Acta. 136: 27- 35.

Zinn, R. A., E. Alvarez, M. Mendez, M. Montaño, E. Ramirez, and Y. Shen. 1997. Influence of dietary sulfur concentration on growth performance and digestive function in feedlot steers. J. Anim. Sci. 75:1723-1728.

Table 4.1 Composition of the diet fed to ruminally cannulated steer donor of ruminal fluid

Ingredient	%, DM
Ground corn	49.1
Alfalfa hay	40.0
Corn steep liquor	5.3
Supplement [1]	5.6
Nutrients, % DM	
CP	13.3
NDF	18.8
S	0.19
K	0.70
Ca	0.70

[1] Formulated to provide 300 mg/day monensin, 90 mg/day tylosin, 2,200 IU/kg vit. A, 22 IU/kg vit. E, 0.3% salt, 10 mg/kg Cu, 0.15 mg/kg Co, 0.5 mg/kg I, 60 mg/kg Mn, 0.25 mg/kg Se, and 60 mg/kg Zn.

Table 4.2 Effects of S titration on *in vitro* VFA and ammonia concentrations, and IVDMD by ruminal microorganisms

	Ground corn – soybean meal							Ground corn – dried distillers grains								*F*-test *P*- values[a]		
Item	0	0.1S	0.2S	0.3S	0.4S	0.5S	0.6S[b]	0	0.1S	0.2S	0.3S	0.4S	0.5S	0.6S	SEM	Sub	S	Sub ×S
IVDMD, %	64.0	63.9	64.2	64.9	65.8	64.8	65.1	60.1	61.6	61.5	61.8	62.0	61.5	60.9	0.74	< 0.01	0.30	0.86
Ammonia, m*M*	25.4	25.7	25.5	25.2	25.2	25.1	24.4	19.9	20.2	19.7	20.1	19.9	20.6	20.6	0.73	< 0.01	0.88	0.10
Acetate, m*M*	63.4	64.7	70.6	66.9	67.1	64.0	71.1	63.4	65.1	63.8	62.2	63.2	63.3	61.8	3.50	< 0.01	0.32	0.02
Propionate, M*m* [Q, C]	42.4	41.7	41.3	41.6	41.5	42.9	40.3	39.5	37.7	38.8	39.9	39.3	39.8	38.6	0.94	< 0.01	0.05	0.54
Butyrate, m*M*	14.0	14.1	13.8	14.0	13.7	13.8	13.4	12.9	12.3	12.6	12.6	12.2	12.9	12.7	1.42	< 0.01	0.33	0.32
Isovalerate, m*M*	1.93	1.99	2.18	2.09	2.02	1.99	2.19	2.14	2.19	2.09	2.01	2.00	2.16	2.07	0.07	0.24	0.35	0.01
Valerate, m*M* [Qt]	1.47	1.44	1.47	1.48	1.43	1.52	1.42	1.64	1.56	1.60	1.65	1.60	167	1.62	0.08	< 0.01	0.02	0.77
Total VFA, m*M*	124.3	124.9	130.5	127.1	126.8	125.3	129.5	121.6	120.0	119.9	119.4	119.4	120.9	118.0	4.67	< 0.01	0.76	0.08
Acetate:Propionate	1.50	1.55	1.72	1.62	1.62	1.50	1.77	1.63	1.73	1.66	1.65	1.62	1.60	1.60	0.11	0.62	0.09	0.03

[a] Sub: effect of substrate type

S: effect of sulfur concentration

Sub ×S: interaction between substrate type and sulfur concentration

[b] 0.1S, 0.2S, 0.3S, 0.4S, 0.5S, and 0.6S represent 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6% S (DM), respectively

[Q] quadratic effect of S concentration, $P = 0.05$

[C] cubic effect of S concentration, $P = 0.05$

[Qt] quartic effect of S concentration, $P = 0.006$

Table 4.3 Effects of S titration on *in vitro* total gas production by ruminal microorganisms and gas composition

	Ground corn – soybean meal				Ground corn – DDGS					*F*-test *P*- Values[b]						
Item	0	0.2S	0.4S	0.6S[a]	0	0.2S	0.4S	0.6S	SEM	Sub.	S	T	Sub. × S	S × T	Sub × T	Sub × S × T
8 h																
Gas production, mL	149.3	130.4	145.0	148.8	155.4	146.2	140.9	142.0	12.54	0.02	0.35	< 0.01	0.63	0.09	< 0.01	0.75
CH_4, %	5.8	5.4	5.3	6.0	5.5	5.2	5.4	6.2	0.52	0.12	0.99	< 0.01	0.14	0.20	0.45	0.78
H_2S, mg/ L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0								
16 h																
Gas production, mL	307.0	286.5	299.5	313.8	284.2	274.8	258.5	274.7	12.54							
CH_4, %	6.8	7.8	6.6	6.3	6.1	5.9	6.8	6.0	0.52							
H_2S, mg/ L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0								
24 h																
Gas production, mL	369.3	353.4	368.3	379.0	337.0	324.9	329.3	334.5	12.54							
CH_4, %	8.3	8.0	7.4	7.8	7.3	6.9	8.4	7.5	0.52							
H_2S, mg/ L	315.0	310.1	290.6	268.2	244.7	290.5	356.9	342.1	187	0.70	0.80		0.40	-	-	-

[a] Sub: effect of substrate type

S: effect of sulfur concentration

T: effect of fermentation time

Sub × S: interaction between substrate type and sulfur concentration

S × T: interaction between sulfur and fermentation time

Sub × T: interaction between substrate type and fermentation time

Sub × S × T: Interaction between substrate type, sulfur concentration, and fermentation time

[b] 0.2S, 0.4S, and 0.6S represent 0.2, 0.4, and 0.6% S (DM), respectively

Figure 4.1 Effects of 0, 0.2, 0.4, or 0.6 % S on total gas production over a 24-h incubation period.

No time × sulfur interaction occurred ($P = 0.09$), but there was an effect of fermentation time ($P < 0.0001$); SEM = 9.1

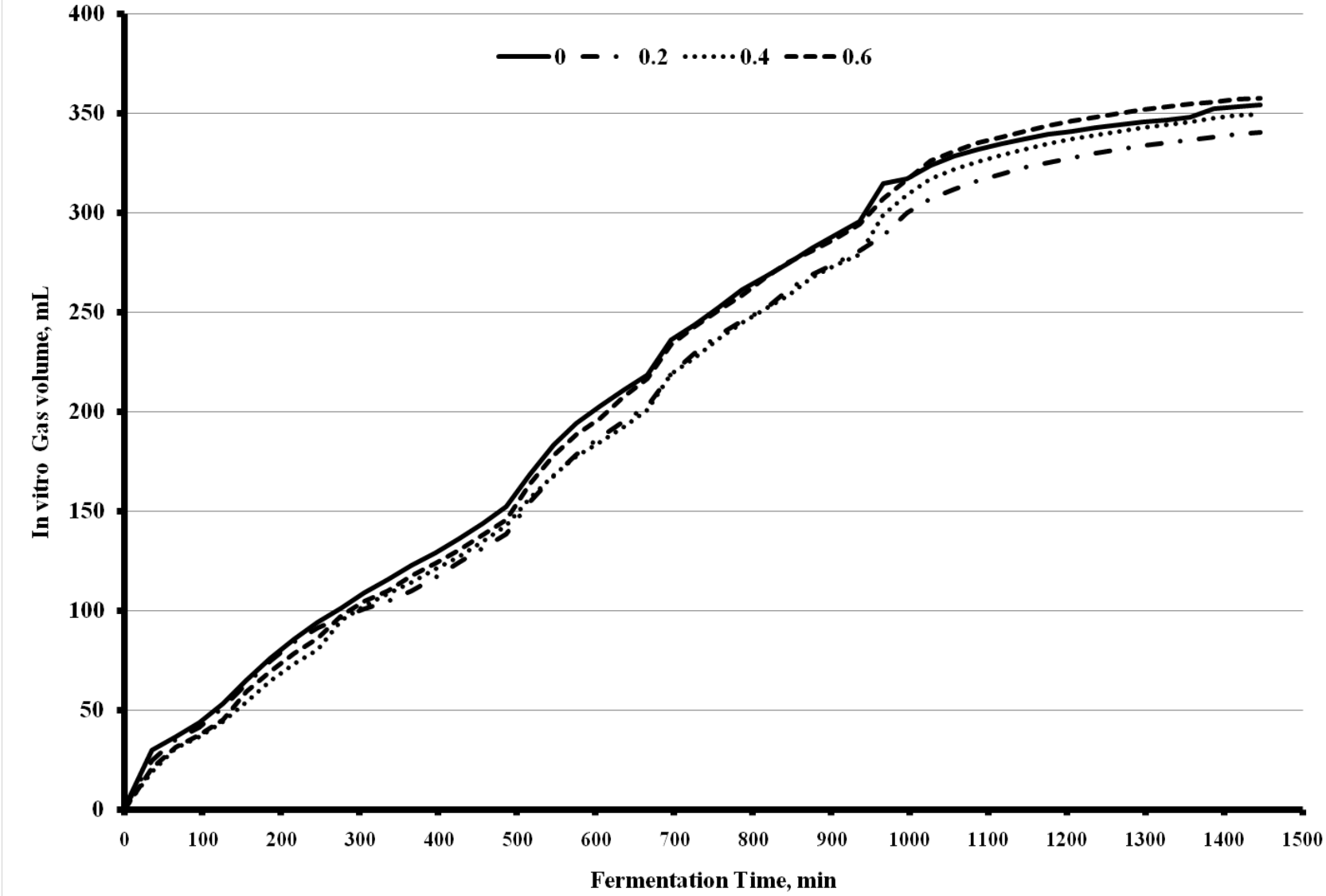
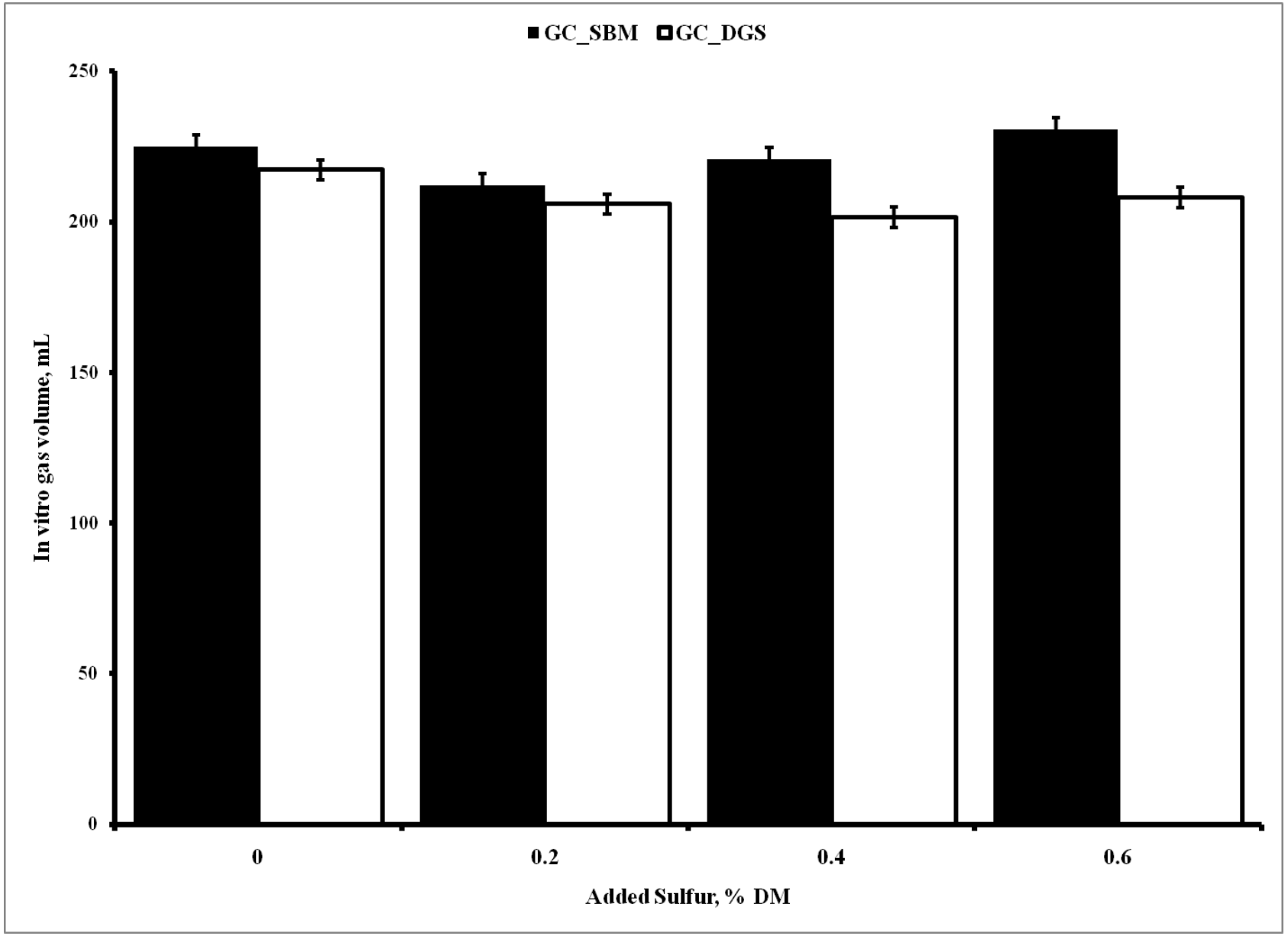

0
0.2
0.4
0.6
In vitro Gas volume, mL
Fermentation Time, min
400
350
300
250
200
150
100
50
0
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500

Figure 4.2 Effects of S titration in substrates based on ground corn and DDGS (GC-DDGS) or ground corn plus urea plus soybean meal (GC-SBM) on *in vitro* total gas production.

Average values over all times are plotted and error bars represent standard error of means. There was no substrate type × sulfur concentration interaction ($P = 0.63$), effect of sulfur ($P = 0.35$), but there was an effect of substrate ($P = 0.02$).


GC_SBM
GC_DGS
250
200
150
100
50
0
In vitro gas volume, mL
0
0.2
0.4
0.6
Added Sulfur, % DM

Chapter 5 - Effects of sporadic feeding of DDGS containing varying concentrations of dietary S on growth performance and carcass characteristics of feedlot cattle

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Abstract

Crossbred yearling steers (n = 50; 462 ± 26.6 kg BW) were used in a finishing trial to evaluate feedlot performance and carcass characteristics when continuously or intermittently exposed to dried distillers grains with solubles (DDGS) containing high S levels. The study was a randomized block design with 3 treatments: chronic high S (CHS; 0.60% S DM), chronic intermediate S (CIS; 0.50% S DM), and sporadic intermediate S (SIS; oscillating from 0.40 or 0.60% S, DM basis). Two DRC-based finishing diets (0.40 and 0.60% S) containing 35% DM of DDGS were mixed each morning. The CIS diet was made by mixing (50:50) 0.40 and 0.60% S diets. The SIS treatment consisted of intermittent feeding of either 0.40 or 0.60% S based on a random feeding schedule. The CIS and SIS treatments delivered the same S content over the entire study period. Steers were blocked by weight and randomly assigned within block to treatments and 50 individual concrete surfaced pens equipped with feed bunks and water fountains that allowed free access to feed and clean water. Steers were fed once daily at approximately 0800 h and feed refusals were determined at approximately 0700 h the following day, thus making it possible to determine actual daily DMI. Steers were harvested on d 100 (n = 27) and 135 (n = 23). Steers fed CHS had 11.2 and 6.1% less ($P < 0.05$) DMI than steers fed CIS and SIS, respectively, but there were no treatment effects on ADG, G:F, or carcass characteristics ($P > 0.10$). High sulfur decreases feed intake, but infrequent exposure to high levels is no more harmful than continuous exposure.

Key words: DMI, feedlot, distillers grains, sulfur

Introduction

Distillers grains with solubles (DGS) are commonly included into finishing diets as an alternative energy and protein source due to their competitive price. However, producers are concerned about inconsistency of S content of DGS within and across ethanol plants (Buckner et al., 2011). According to Kerr et al. (2008), S content of DGS may be over 9 g/kg. Ruminal microorganisms metabolize S to synthesize S-containing amino acids and B-vitamins (NRC, 2000), and also produce sulfide (S^-), either by reducing inorganic S, degrading S-containing amino acids, or oxidizing elemental S (Bray and Till, 1975). Ruminal S^- can then be absorbed

across ruminal wall. Non absorbed S^- bind to H^+ to form H_2S that is eructated and can potentially cause polioencephalomalacia (PEM) when aspirated into the lungs (Gould, 1998).

Besides the PEM risk, elevated dietary S has been reported to decrease cattle feedlot performance (Zinn et al., 1997; Spears et al., 2011). In a study by Loneragan et al. (2001), increasing water S concentrations depressed DMI by feedlot steers during the first 28 days on feed, after which feed consumption improved and remained relatively stable until the end of the study. Loneragan et al. (2001) suggested that the observed improvement of DMI over time could be attributable to upregulated capacity of the liver to detoxify elevated amounts of absorbed sulfide. Uwituze et al. (2011) reported improvement of DMI by steers fed 0.65% S DM, as time on feed progressed. We hypothesized that infrequent exposure to elevated S could be more detrimental than chronic exposure due to the inability of animals to adapt within short intervals of time. The objective of this study was to evaluate feedlot performance of cattle when exposed to dried distillers grains with solubles (DDGS) containing high S levels, either continuously or intermittently.

Materials and Methods

Animal care and handling described herein followed procedures approved by the Kansas State University Institutional Animal Care and Use Committee protocol no. 2615.

Treatments

Two dry-rolled corn (DRC)-based finishing diets (0.4 and 0.6% S) containing 35% DM of DDGS were used to make 3 experimental treatments: 1) continuous high S (CHS; 0.6% DM), 2) continuous intermediate S (CIS; 0.5% DM), and 3) sporadic intermediate S (SIS; 0.4 or 0.6% DM). The CIS and SIS treatments were designed to deliver similar amounts of S over the entire study period. The CIS treatment was made by mixing (50:50) 0.4 and 0.6% S diets, whereas the SIS treatment consisted of alternating back and forth between 0.4 and 0.6% S diets based on a random daily feeding schedule. Assuming a feeding period of 140 d (Uwituze et al., 2011), a random number was assigned to each d, and the lowest 70 randomly generated numbers were allocated to the 0.4% S diet and the highest 70 randomly generated numbers to 0.6% S diet. Consequently, there were intervals of various length in d during which steers were fed the 0.6% S diet, and then abruptly switched to the 0.4% S diet, and vice versa (Figure 5.1). The advantage

is that there was no regular pattern of S administration, which is more likely to emulate conditions encountered by large commercial feedlots, where in most instances a single load of DGS is used in a single day.

The 0.4% S concentration consisted of S contents of individual ingredients without added H_2SO_4. Dried DGS were mixed with H_2SO_4 [983.2 kg of DDGS + 16.2 kg of H_2SO_4 (93% concentration)] prior to mixing with other ingredients to obtain the 0.6% S concentration. Sulfuric acid was used as a source of S to emulate actual use in ethanol plants. One ton of DDGS treated with $H_2SO_4^-$ was mixed each wk. Two loads of DDGS were received for the entire 135-d study period from Poet Nutrition (Sioux Falls, SD) and S concentrations were similar for the 2 loads (0.91%, Table 5.1).

Concentrations of S were determined based on a study conducted previously at Kansas State University, in which steers fed diets with 0.65% S tended to increase feed intake as they adapted to S over time (Uwituze et al., 2011), with some modifications. The 0.6% S concentration was utilized in the present study, instead of 0.65% S, to allow cumulative S intakes that were comparable between CIS and SIS groups over a 140-d feeding period. Animals harvested on d 100 were fed diets containing 0.4% S for 49 d and diets containing 0.6% S for 50 d. Animals harvested on d 135 were fed diets containing 0.4% S for 69 d and diets containing 0.6% S for 65 d.

Animals and Feeding Management

Crossbred yearling steers were fed ground alfalfa hay *ad libitum* and had free access to municipal water upon arrival at the Kansas State University feedlot. One day after arrival, each steer was weighed and identified with two ear tags displaying a unique number for each steer. Steers also were vaccinated against clostridial diseases using Vision-7 (Intervet/Schering-Plough Animal Health, Summit, NJ), treated for parasites using Safe-Guard (Intervet/Schering-Plough Animal Health), and Ultra-Boss (Intervet /Schering-Plough Animal Health), and implanted with Revalor-XS (Intervet/Schering-Plough Animal Health). Steers were subsequently fed a high-roughage diet containing (DM basis) 60% corn silage, 27% DDGS (Poet Nutrition, Sioux Falls, SD), 9.2% DRC, and 3.8% supplement for 30 d before initiation of the study.

Before initiation of the study, 50 steers were individually weighed (462 ± 26.6 kg BW) and grouped in weight strata (16 of 3 steers each, and 1 with only 2 steers). Steers were then

randomly assigned within weight strata to treatments and concrete-surfaced individual feeding pens. Pens were located inside 4 barns (20 pens per barn) open at the south end representing 40% of the pen. Each pen was 1.5 m wide × 6 m long, and was equipped with a feed bunk and water fountain that allowed free access to feed and clean water. Though there were 2 full barns (20 steers/ barn) and 2 with only 5 steers per barn, all treatments were represented in each barn.

Steers were adapted gradually to finishing diets (Table 5.2) through four 5-d step-up diets. During the step-up period, corn silage was decreased in 12.5% (DM) increments and was replaced with DRC in increments of 10.5% (DM) and untreated DDGS in 2% (DM) increments for the 0.4% S diet. For the 0.6% S diet, corn silage and untreated DDGS were gradually replaced with DRC in 10.3% (DM) increments and acid-treated DDGS in increments of 8.9% (DM). With respect to the 0.5% S diet, corn silage and untreated DDGS decreased in increments of 12.5% (DM), and 2.1% (DM), respectively, whereas DRC and acid-treated DDGS increased in 10.4 and 4.2% (DM) increments, respectively. Experimental diets were mixed daily and each steer was fed *ad libitum* around 1000 h. Weights of fresh feed provided were recorded daily and feed refusals were removed and weighed the following morning before feeding, thus making it possible to determine actual daily DMI (Figure 5.1).

General health and clinical signs of polioencephalomalacia were recorded daily from the beginning of the study until harvest. Steers were weighed in 28-d intervals. The 9 heaviest steers per treatment were harvested on d 100 (n = 27). The remaining steers (n = 23) were fed 8.33 mg of Zilpaterol-HCl /kg of diet DM for 20 d followed by a 3-d withdraw before harvest on d 135. Final shrunk BW (gross BW × 0.96) were determined immediately before cattle were shipped 451 km to a commercial abattoir in Holcomb, KS for both harvest times.

Sampling and Laboratory Analyses

All feedstuffs and municipal drinking water were analyzed for S concentration (AOAC, 1990, official method 923.01) before formulation of the experimental diets. Feedstuffs were sampled weekly, and proximate analyses were completed for monthly composited samples as described by Uwituze et al. (2010). Briefly, DM was determined according to Undersander et al. (1993). Ether extract was performed following AOAC (1995) official method 920.39. Starch contents were analyzed according to Herrera-Saldana and Huber (1989) and Gochman and Schmitz (1972) using a Technicon Autoanalyzer III (SEAL Analytical, Mequon, WI), and an

ANKOM fiber analyzer (ANKOM Technology Corp., Fairport, NY) was used to measure NDF contents following procedures described by Van Soest et al. (1991). Crude protein contents were computed based on N contents determined using a LECO FP–2000 nitrogen analyzer (LECO Corp., St Joseph, MI) according to AOAC (1995) official method 990.03.

Carcass Traits

On both harvest days (d 100 and d 135), HCW were obtained, and incidence and severity of liver abscess scores were recorded according to Brink et al. (1990). Measurements for subcutaneous fat thickness over the 12th rib, percent KPH, and LM area were taken after chilling carcasses for 24 h, and marbling scores, yield grades, and quality grades were determined by USDA graders. Final BW were adjusted for gut fill by dividing HCW by a 63.5% dressed yield, which is the baseline used for the grid system under which the steers were marketed. This adjustment was possible because carcass trim was negligible for steers in the current study. Average daily gains were computed by subtracting initial live BW from carcass-adjusted final BW, and dividing the result by days on feed (DOF). Feed conversions were calculated by dividing ADG by daily DMI. Dressing percentage was determined as HCW divided by final shrunk BW.

Statistical Analyses

Data were analyzed using the Mixed procedure of SAS version 9.2 (SAS Inst. Inc., Cary, NC) for a randomized block design with 2 harvest times (early = 100 d vs. late = 135 d) and 3 dietary treatments. Steer was the experimental unit and barn was the blocking factor.

First, effects on DMI over time were analyzed as repeated measures using the Mixed procedure of SAS to check whether harvest interacted with treatments and DOF. Steer was the experimental unit, and the model statement included treatment, harvest time, DOF, interaction between treatment, harvest time, and DOF, and all 2-way interactions. The random effects were barn and steer within barn, harvest time, and treatment.

Second, the effects of treatment and DOF within each harvest group were tested as repeated measures using the Mixed procedure of SAS. The model statement included treatment, DOF, and interaction between treatment and DOF and random effects consisted of barn and steer within barn, and treatment.

Third, the slice option with F-protected LSMEANS was used to compare means of the three treatments at each day (where day is the 'slice'). Separation of treatment means by day was accomplished using pairwise comparisons.

To evaluate differential effects of feeding 0.4 or 0.6% S (DM basis) on feed consumption within the SIS group, DMI was averaged across DOF for 0.4% S vs. 0.6% S for each animal and means were analyzed using the Mixed procedure of SAS. The model statement included feeding schedule and harvest × schedule interaction, and random effects consisted of barn and steer within barn.

Treatment effects on growth performance and carcass characteristics were analyzed using the Mixed procedure of SAS version 9.2. Experimental unit was the steer and random effects were barn and steer nested within barn and harvest time. The model statement included effects of treatment, harvest time, and the interaction between treatment and harvest time. Treatment means for non-categorical data were determined using the F-protected LSMEANS procedure of SAS, and significance was declared at $P \leq 0.05$. Liver abscess scores, USDA yield grades, and USDA quality grades were analyzed using a Chi-square test with the "exact" option due to low occurrences. A $P \leq 0.05$ was declared significant.

Results

Sulfur levels in drinking water were not detectable (data not shown). Composition of DDGS used in the current study is presented in Table 5.1. None of the steers on study exhibited symptoms of PEM or any other diseases.

Treatment Effects on Feed Consumption Over Time

No harvest time × DOF × treatment interaction occurred ($P > 0.10$) with respect to DMI, but there was an interaction between harvest time and treatment ($P < 0.001$). Therefore, data pertaining to DMI over time will be discussed within harvest groups. For the group harvested at d 100 (n = 27), there was a treatment by DOF interaction ($P < 0.01$) and means comparison indicated that after d 11, treatment means for DMI were different ($P < 0.05$) with few isolated exceptions (Figure 5.1). Steers fed CIS had consistently greater DMI ($P < 0.05$) than steers fed CHS from d 12 through 100 DOF. Dry matter intake by steers fed SIS was similar ($P > 0.10$) to that of steers fed CIS except when the SIS group was fed a 0.6% S diet on d 13, 16, 18, 19, and

21 ($P < 0.05$). For the group harvested on d 135 (n = 23), DMI was not affected ($P > 0.10$) by treatment × DOF interaction, treatment, or DOF (Figure 5.2).

Comparison between feeding 0.6% and 0.4% S within the SIS group revealed no differences ($P = 0.20$; Figure 5.3) in DMI when averaged over days on which steers were fed 0.6% S or 0.4%. On average, steers consumed 11.4 kg DM/d on days they were fed diets containing 0.4% S and 11.3 kg/d on d they were fed diets containing 0.6% S.

Growth Performance

There were no interactions between treatment and harvest time ($P > 0.85$) for measures of growth performance (Table 5.3), hence only treatment effects will be discussed. Steers fed CIS had 11.2% greater ($P < 0.01$) DMI than CHS but similar ($P = 0.13$) DMI to SIS group. Steers fed SIS and CHS had similar DMI ($P = 0.12$). There were no treatment effects with respect to carcass-adjusted ADG ($P = 0.30$) or G:F ($P = 0.73$), but steers fed CIS and SIS tended ($P = 0.06$) to have greater shrunk final BW than steers fed CHS (Table 5.3). Carcass-adjusted final BW were not statistically different among treatments ($P = 0.29$), but carcass-adjusted final BW of steers fed CHS were 15 and 16 kg less compared to steers fed SIS or CIS, respectively.

Carcass Data

Data for carcass characteristics are presented in Table 5.4. Treatments had no effects on HCW ($P = 0.29$), dressing percent ($P = 0.11$), LM area ($P = 0.74$), 12th rib subcutaneous fat thickness ($P = 0.49$), percent KPH ($P = 0.14$), marbling scores ($P = 0.11$), liver abscess scores ($P = 0.59$), USDA quality grades ($P \geq 0.18$), or USDA yield grades ($P = 0.97$). Steers fed CHS had numerically lighter carcasses, lower KPH, less marbling, and fewer carcasses grading Choice compared with steers fed SIS.

Discussion

Sulfate is an essential mineral, not only for ruminal microbial populations to synthesize B-vitamins and S-containing amino acids (Bray and Till, 1975), but also for the ruminant itself for various physiological purposes such as mucus secretion, and involvement in regulation of various metabolic pathways (Bird, 1972). Excessive S intake can have deleterious effects on health and growth performance of cattle (Raisbeck, 1982; Kandylis, 1984). The current study

suggests that elevated dietary S decreases feed intake, but infrequent exposure to high levels is no more harmful than continuous exposure. Steers fed CIS had greater DMI ($P < 0.05$) than steers fed CHS from 12 through 100 DOF. Feeding 0.4 or 0.6% S alternatively within the SIS group had no effect ($P > 0.10$) on DMI. The remaining measures of growth or carcass characteristics were not affected by treatments ($P > 0.10$).

We previously reported that feeding 0.65% S decreased DMI by 8.9% and ADG by 12.9% compared with feeding 0.42% S, but dietary S concentration had no effect on G:F (Uwituze et al., 2011). Hot carcass weights, KPH, and yield grades also decreased as a result of feeding 0.65 rather than 0.42% S DM (Uwituze et al., 2011). A number of studies reported decreased DMI with increasing S concentrations with various effects on other measures of growth performance and carcass characteristics.

In a study by Loneragan et al. (2001), increasing water S concentrations depressed DMI by feedlot steers during the first 28 DOF after which feed consumption improved and remained relatively unchanged until the end of the study. Uwituze et al. (2011) reported improvement of DMI by steers fed 0.65% S DM, especially from 49-77 DOF. Loneragan et al. (2001) suggested that the observed improvement of DMI over time could be attributable to enhancement of the liver capacity to detoxify elevated amounts of S^- absorbed during early DOF.

Spears et al. (2011) reported that ADG of steers fed 0.46% S in finishing diets based on ground corn was lesser than that of steers fed 0.12 or 0.31% S, but G:F was not affected by dietary S levels. Loneragan et al. (2001) observed decreased ADG and G:F with increasing S content in drinking water whereas ADG and G:F responded quadratically to increasing dietary S in 0.05% increments from 0.15, to 0.25% S (DM) with the optimal concentration of 0.20% (Zinn et al., 1997). Pendlum et al. (1976) observed no effect on growth performance of finishing Holstein steers fed 0.11, 0.26, or 0.45% dietary S in ground corn-based diets. According to Rumsey (1978), adding 0, 0.14, and 0.42% of sublimed S in cracked corn-based diets (0.14% S in basal diet) had no effect on ADG, but decreased DMI and, as a result, improved feed efficiency of beef steers.

Some researchers have attributed deleterious effects of high S intake on DMI to inhibition of gut motility. Kandylis (1984) suggested DMI decreases as a result of inhibition of ruminal and intestinal motility when excessive H_2S is produced in the rumen. In a study by Bird

and Moir (1971), sheep ruminally infused with 6 g/d of S as sulfate stopped eating or drinking, but sheep infused with the same amount of S duodenally did not decrease feed intake.

Other scientists suggest DMI may decrease as ruminal microorganisms adapt to elevated dietary S (Huisingh et al., 1974; Loneragan et al., 2001). In research by Cummings et al. (1995), sulfate-reducing bacteria increased their capacity to produce ruminal H_2S after 8 to 12 d exposure to high dietary S. We previously observed a negative correlation between ruminal H_2S concentration and DMI *in vivo* (Uwituze et al., 2011). Using fluorescence *in situ* hybridization, Dar et al. (2008) reported changes in bacterial populations due to variations of lactate to sulfate molar ratios. However, recent *in vitro* studies indicated no S effect on fermentative activity of ruminal microorganisms (Quinn et al., 2009; Smith et al., 2010).

The decreased DMI relative to increasing dietary S also may relate to lower dietary cation-anion difference (DCAD; Tucker et al., 1991; Spears et al, 2011). Increasing DCAD level from 30 to 0 milliequivalents (mEq)/100 g DM improved DMI by dairy cows (Tucker et al., 1991), but Ross et al. (1994) and Spears et al. (2011) found no improvement of feed intake in finishing cattle when they increased DCAD from 0 to 15 mEq/100 g DM and from -16.6 to -2.2 mEq/100 g DM, respectively. Using a DCAD equation of mEq $(Na + K) - (Cl^- + S^{-2})$/100 g DM, DCAD values in the present study were -6.0, -13.0, and -20.9 mEq/100 g DM for diets containing 0.4, 0.5, and 0.6% S respectively. Dietary cation-anion difference values calculated for 0.4 and 0.5% S diets in this study were similar to those reported by Spears et al. (2011) when they fed 0.15 and (-7.2 mEq/100 g DM) and 0.31% (-16.6 mEq/100 g DM) dietary S in ground corn-based finishing diets, suggesting lack of S effect on DCAD in the present study.

Feeding DGS has been reported to increase dietary S concentration (Corrigan et al., 2009, Nichols et al., 2011), thus causing health problems and poor growth performance and carcass characteristics. In a study by Wilken et al. (2009), 6% of cattle fed diets containing 0.47 to 0.59% S DM from blends of WDGS and wet corn gluten feed were diagnosed with PEM. Moreover, 15% of steers fed 50% DDGS (with 0.60% S) in a DRC-based diet showed symptoms related to S toxicity (Buckner et al., 2007). Although higher or similar levels of S were fed in the current study, no steers were diagnosed with PEM or other digestive disorders. Unlike in studies by Buckner et al. (2007) and Wilken et al. (2009), steers in this study were individually fed. This may have allowed animals to control their intake without the pressure of competition from their peers, thus decreasing the likelihood of digestive disorders and PEM.

Though steer in the current study exhibited no signs of S toxicity, steers fed CHS had 11.2 and 6.1% less DMI than steers fed CIS and SIS, respectively. Sarturi et al. (2011) evaluated effects of 0.33, 0.43, and 0.54 % S from WDGS or DDGS on performance of finishing steers. Their results indicated that increasing dietary S decreased DMI more so with WDGS than DDGS, but WDGS improved G:F.

We hypothesized that infrequent exposure to elevated S could be more deleterious than chronic exposure due to the inability of animals to adapt within short intervals of time. Results from this study indicated that feeding elevated dietary S continuously decreased DMI, but the deleterious effects of elevated S (0.6% S DM) were not exacerbated with infrequent exposure.

References

AOAC. 1990. Official Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 1995. Official Methods of Analysis. 16th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Bird, P. R. 1972. Sulphur metabolism and excretion studies in ruminants X. Sulphide toxicity in sheep. Aust. J. Biol. Sci. 25:1087-1098.

Bird, P. R., and R. J. Moir. 1971. Sulphur metabolism and excretion studies in ruminants. I. The absorption of sulphate in sheep after intraruminal or intraduodenal infusions of sodium sulphate. Aust. J. Biol. Sci. 24:1319-1328.

Bray, A. C., and A. R. Till. 1975. Metabolism of sulphur in the gastro-intestinal tract. Pages 243-260 in Digestion and Metabolism in the Ruminant. I.W. McDonald and A. C. I. Warner, ed. Univ. New England Publ. Unit. Armidale.

Brink, D. R., S. R. Lowry, R. A. Stock and J. C. Parrott. 1990. Severity of liver abscesses and efficiency of feed utilization of feedlot cattle. J. Anim. Sci. 68:1201-1207.

Buckner, C. D., T. L. Mader, G. E. Erickson, S. L. Colgan, K. K. Karges, and M. L. Gibson. 2007. Optimum concentrations of dry distillers grains with solubles for finishing beef steers. Beef Cattle Report MP 90. Pages 36-38. University of Nebraska, Lincoln.

Buckner, C.D., M. F. Wilken, J. R. Benton, S. J. Vanness, V. R. Bremer, T. J. Klopfenstein, P. J. Konoff, and G. E. Erickson. 2011. Nutrient variability for distillers grains plus solubles and dry matter determination of ethanol by-products. Prof. Anim. Sci. 27:57-64.

Corrigan, M. E., G. E. Erickson, T. J. Klopfenstein, M. K. Luebbe, K. J. Vander Pol, N. F. Meyer, C. D. Buckner, S. J. Vanness, and K. J. Hanford. 2009. Effect of corn processing method and corn wet distillers grains plus solubles inclusion concentration in finishing steers. J. Anim. Sci. 87: 3351-3362.

Cummings, B. A., D. H. Gould, D. R. Caldwell, and D. W. Hamar. 1995. Ruminal microbial alterations associated with sulfide generation in steers with dietary sulfate-induced polioencephalomalacia. Am. J. Vet. Res. 56:1390–1395.

Dar, S. A., R. Kleerebezem, J. G.Kuenen, and G. Muyzer. 2008. Competition and coexistence of sulfate –reducing bacteria, acetogens and methanogens in a lab-scale anaerobic bioreactor as affected by changing substrate to sulfate ratio. Appl. Microbiol. Biotechnol. 78:1045-1055.

Gochman, N., and J. M. Schimitz. 1972. Application of new peroxide indicator reaction to specific automated determination of glucose with glucose oxidase. Clin. Chem. 18:943-950.

Gould, D. H. 1998. Polioencephalomalacia. J. Anim. Sci. 76:309-314.

Herrera-Saldana R., and J. T. Huber. 1989. Influence of varying protein and starch degradabilities on performance of lactating cows. J. Dairy Sci. 72:1477-1483.

Huisingh, J., J. J. McNeill, and G. Matrone. 1974. Sulphate reduction by a *Desulfovibrio* species isolated from sheep rumen. Appl. Microbiol. 28:489-497.

Kandylis, K. 1984. Toxicology of S in ruminants: Review. J. Dairy Sci. 67:2179–2187.

Kerr, B. J., C. J. Ziemer, T. E. Weber, S. L. Trabue, B. L. Bearson, C. C. Shurson, and M. H. Whitney. 2008. Comparative sulfur analysis using thermal combustion or inductively coupled plasma methodology and mineral composition of common livestock feedstuffs. J. Anim. Sci. 86:2377-2384.

Loneragan, G. H., J. J. Wagner, D. H. Gould, F. B. Garry, and M.A. Thoren. 2001. Effects of water sulfate concentration on performance, water intake and carcass characteristics of feedlot steers. J. Anim. Sci. 79:2941-2948.

Nichols, C. A., K. M. Rolfe, C. D. Buckner, G. E. Erickson, T. J. Klopfenstein. 2011. Effect of source and level of sulfur on rumen metabolism and finishing performance. Beef Cattle Report MP 94. Pages 60-61. University of Nebraska, Lincoln.

NRC. 1984. Nutrient Requirement of Beef Cattle. 6th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th ed. National Academy Press, Washington, D. C.

Pendlum, L. C., J. A. Boling, and N. W. Bradley. 1976. Plasma and ruminal constituents and performance of steers fed different nitrogen sources and concentrations of sulfur. J. Anim. Sci. 43:1307-314.

Quinn, M. J., M. L. May, K. E. Hales, N. DiLorenzo, J. Leibovich, D. R. Smith, and M. L. Galyean. 2009. Effects of ionophores and antibiotics on *in vitro* hydrogen sulfide production, dry matter disappearance, and total gas production in cultures with a steam-flaked corn-based substrate with or without added sulfur. J. Anim. Sci. 87:1705–1713.

Raisbeck, M. F. 1982. Is polioencephalomalacia associated with high sulfate diets? J. Am. Vet. Med. Assoc. 180:1303-1305.

Ross, J. G. W. Spears, and J. D. Garlich. 1994a. Dietary electrolyte balance effects on performance and metabolic characteristics in finishing steers. J. Anim. Sci. 72:1600-1607.

Rumsey, T. S. 1978. Effects of dietary S addition and Synovex- S ear implants on feedlot steers fed an all-concentrate finishing ration. J. Anim. Sci. 46:463-477.

Sarturi, J. O., G. E. Erickson, T. J. Klopfenstein, J. T. Vasconcelos, J. R. Benton, W. A. Griffin. 2011. Effects of sulfur concentration in distillers grains with solubles in finishing cattle diets. Beef Cattle Report MP 94. Pages 62-64. University of Nebraska, Lincoln.

Smith, D. R., N. DiLorenzo, J. Leibovich, M. L. May, M. J. Quinn, J. W. Homm, and M. L. Galyean. 2010. Effects of sulfur and monensin concentrations on *in vitro* dry matter disappearance, hydrogen sulfide production, and volatile fatty acid concentrations in batch culture ruminal fermentations. J. Anim. Sci. 88:1503–1512.

Spears, J. W., K. E. Lloyd, and R. S. Fry. 2011. Tolerance of cattle to high dietary sulfur and effect of dietary cation-anion balance. J. Anim Sci. doi:10.2527/jas.2010-3265.

Tucker, W. B., J. F. Hogue, D. F. Watermann, T. S. Siverson, Z. Xin, R. W. Hemken, J. A. Jackson, G. D. Adams, and L. J. Spicer. 1991. Role of sulfur and chloride in the dietary cation-anion balance equation for lactating dairy cattle. J. Anim. Sci. 69:1205-1213.

Undersander, D., R. D. Mertens, and N. Thiex. 1993. Forage Analysis Procedures. Natl. Forage Testing Assoc., Omaha, NE.

Uwituze, S., G. L. Parsons, M. K. Shelor, B. E. Depenbusch, K. K. Karges, M. L. Gibson, C. D. Reinhardt, J. J. Higgins and J. S. Drouillard. 2010. Evaluation of dried distillers grains and roughage source in steam-flaked corn finishing diets. J. Anim. Sci. 88:258-274.

Uwituze, S., G. L. Parsons, C. J. Schneider, K. K. Karges, M. L. Gibson, L. C. Hollis, J. J. Higgins, and J. S. Drouillard. 2011. Evaluation of sulfur content of dried distillers grains with solubles in finishing diets based on steam-flaked corn or dry-rolled corn J. Anim Sci. doi:10.2527/jas.2010-3103.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Wilken, M. F., M. K. Luebbe, G. E. Erickson, T. J. Klopfenstein, and J. R. Benton. 2009. Effects of feeding high concentrations of byproducts in different combinations to finishing steers. Beef Cattle Report MP 92. Pages 76-78. University of Nebraska, Lincoln.

Zinn, R. A., E. Alvarez, M. Mendez, M. Montano E. Ramirez, and Y. Shen. 1997. Influence of dietary sulfur level on growth performance and digestive function in feedlot cattle. J. Anim. Sci. 75:1723-1728.

Table 5.1 Composition of corn dried distillers grains with solubles (DDGS) used in finishing diets

Item, % DM	DDGS	High DDGS[1]
n	6	6
DM	92.5	92.1
CP	27.8	27.5
NDF	27.5	25.7
Ether extract	11.0	10.8
Ca	0.08	0.06
P	0.86	0.84
K	1.00	0.89
S	0.91	1.46

[1]The High S DDGS was obtained by mixing 16.2 kg H_2SO_4 (93% concentration) with 983.2 kg of DDGS to attain 1.46% S.

Table 5.2 Composition of dry-rolled corn-based finishing diets containing dried distillers grains with solubles (DDGS) with various S concentrations

Item	0.4% S	0.5% S	0.6% S
Ingredients, % DM			
n	4	4	4
Dry-rolled corn	44.7	44.5	44.2
DDGS	37.7	20.0	-
High sulfur DDGS	-	17.9	38.1
Corn silage	13.0	13.0	13.0
Supplement [1]	2.3	2.3	2.4
Feed additives [2]	2.3	2.3	2.3
Analyzed composition, % DM			
DM	70.0	70.0	69.9
CP	16.0	16.0	16.0
Degradable intake protein	51.3	51.3	51.3
Ether extract	6.6	6.6	6.6
NDF	19.3	19.0	18.7
Ca	0.7	0.7	0.7
P	0.5	0.5	0.5
K	0.7	0.7	0.7
S	0.40	0.51	0.63
Net energy, Mcal/kg [3]			
Maintenance	2.03	2.01	2.01
Gain	1.37	1.37	1.37
Dietary cation-anion difference (DCAD), mEq/100 g DM [4]	- 6.01	-13.0	- 20.9

[1] Formulated to provide 2,200 IU/kg vitamin A; 22 IU/kg vitamin E; 10 mg/kg Cu; 60 mg/kg Zn; 60 mg/kg Mn; 0.5 mg/kg I; 0.25 mg/kg Se; and 0.15 mg/kg Co (DM basis).

[2] Provided 300 mg/d monensin (Elanco Animal health, Indianapolis, IN) ; 90 mg/d tylosin (Elanco Animal health, Indianapolis, IN), and zilpaterol-HCl (Intervet, Inc., Millsboro, DE), fed for 21 d before harvest at 8.33 mg/kg of diet DM, followed by a 3-d withdraw period.

[3] Calculated using prediction equations from Beef NRC, 1984.

[4] Calculated using equation DCAD = millequivalents (mEq) of (Na + K) - (Cl^- +S) /100 g DM (Tucker et al., 1991).

Table 5.3 Growth performance of finishing steers fed dry-rolled corn diets with continuously 0.6% S (CHS), continuously 0.5% S (CIS), or alternating 0.4 or 0.6% S (SIS)

	Treatments				
Item	CHS	CIS	SIS	SEM	*P*-value
n	17	16	17		
Initial BW, kg	457	460	460	15.2	0.65
Final BW, kg [1]	603	619	618	9.8	0.29
Shrunk BW, kg [2]	582	607	599	8.6	0.06
DMI, kg/d [3]	9.8[a]	10.9[b]	10.4[b]	0.33	0.01
ADG, kg/d [1]	1.34	1.50	1.46	0.074	0.30
G:F [1]	0.136	0.137	0.141	0.006	0.78

[1] Final weight was calculated by dividing carcass weight by a common dressing percentage (63.5%).

[2] Shrunk BW = Final live BW × 0.96

[3] Within a row, numbers bearing different superscripts are different, $P < 0.05$

Table 5.4 Carcass characteristics of finishing steers fed dry-rolled corn diets with continuously 0.6% S (CHS), continuously 0.5% S (CIS), or alternating 0.4 or 0.6% S (SIS)

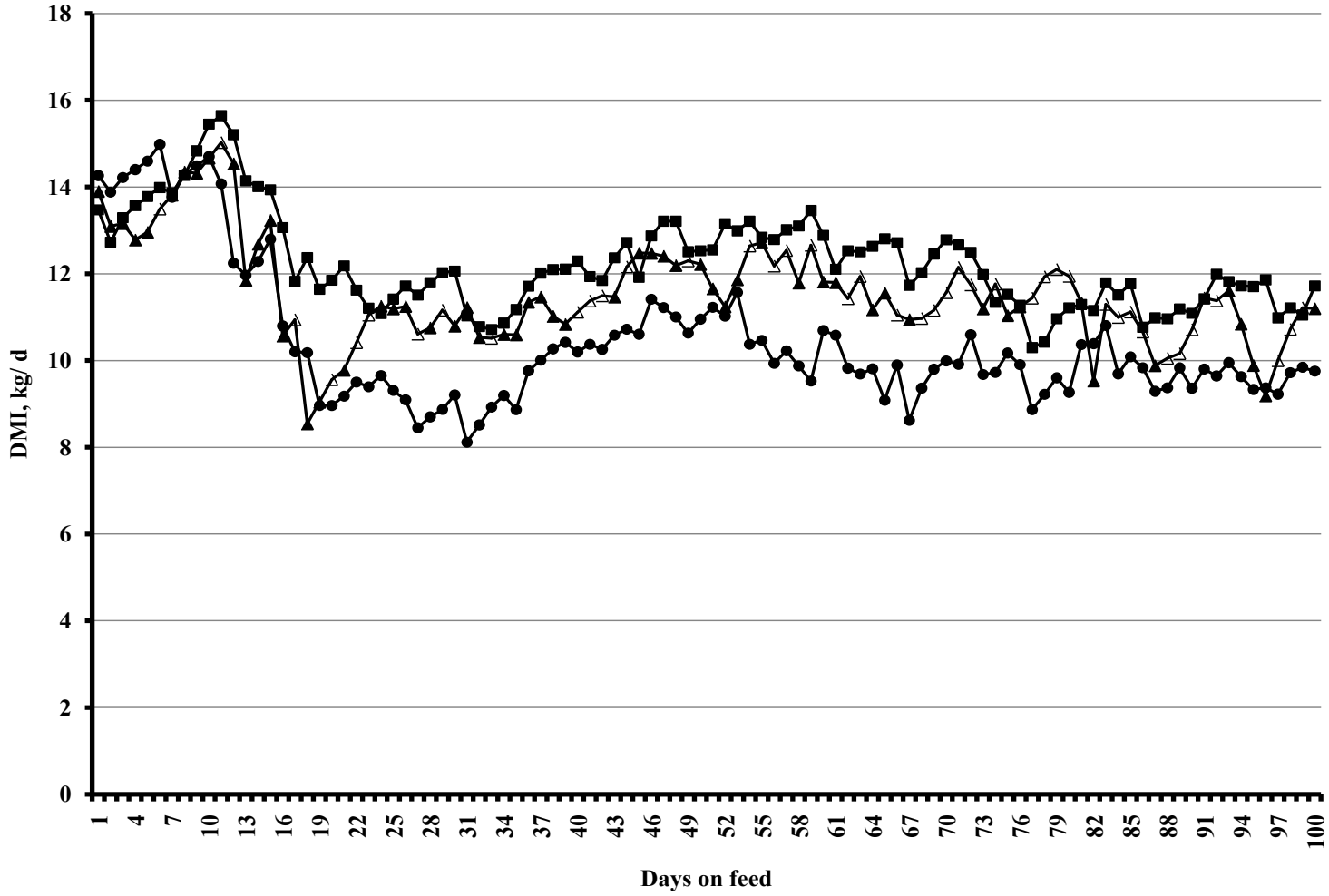
	Treatments				
Item	CHS	CIS	SIS	SEM	*P*-value
n	17	16	17		
HCW, kg	383	393	392	6.2	0.29
Dressing, %	65.9	64.9	65.6	0.5	0.12
LM area, cm^2	94.9	94.5	96.7	2.13	0.74
KPH, %	1.97	1.99	2.25	0.133	0.14
12th rib fat, cm	1.07	1.25	1.23	0.120	0.49
Liver abscess, % [1]	23.5	12.5	23.5	-	0.59
A_+	0	0	0	-	
A_0	17.65	6.25	17.65	-	0.75
A^-	5.8	6.25	5.8	-	0.99
Marbling score [2]	400	446	447	22.8	0.11
Yield grade	2.0	2.1	2.0	0.22	0.97
Choice, % [1]	47.4	68.8	76.5	-	0.18
Select, % [1]	41.5	31.2	23.5	-	0.54
Standard, % [1]	11.1	0	0	-	0.35

[1]Obtained using Chi square test with "exact" option due to low occurrence.

[2] Marbling scores: 400- 499: Small

Figure 5.1 Feed intake for the early havested steers continuously fed 0.6% S (CHS) or 0.5% S (CIS), or sporadically fed 0.4 and 0.6% S (SIS)

Circles represent CHS, triangles represent SIS, and squares represent CIS treatments, respectively. Within the SIS treatment, open triangles indicate days on which steers were fed diets containing 0.4% S, and closed triangles indicate days on which steers were fed diets containing 0.6% S. There was a treatment × feeding time interaction ($P < 0.001$), effect of treatment ($P = 0.0003$), and effect of feeding time ($P < 0.001$); SEM = 0.822


DMI, kg/ d
Days on feed
0
2
4
6
8
10
12
14
16
18
1
4
7
10
13
16
19
22
25
28
31
34
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94
97
100

Figure 5.2 Feed intake for the late havested steers continuously fed 0.6% S (CHS) or 0.5% S (CIS), or sporadically fed 0.4 and 0.6% S (SIS)

Circles represent CHS, triangles represent SIS, and squares represent CIS treatments, respectively. Within the SIS treatment, open triangles indicate days on which steers were fed diets containing 0.4% S, and closed triangles indicate days on which steers were fed diets containing 0.6% S. There was a treatment × feeding time interaction ($P < 0.001$) and effect of feeding time($P < 0.001$), but no effect of treatment ($P = 0.80$), SEM = 0.981.

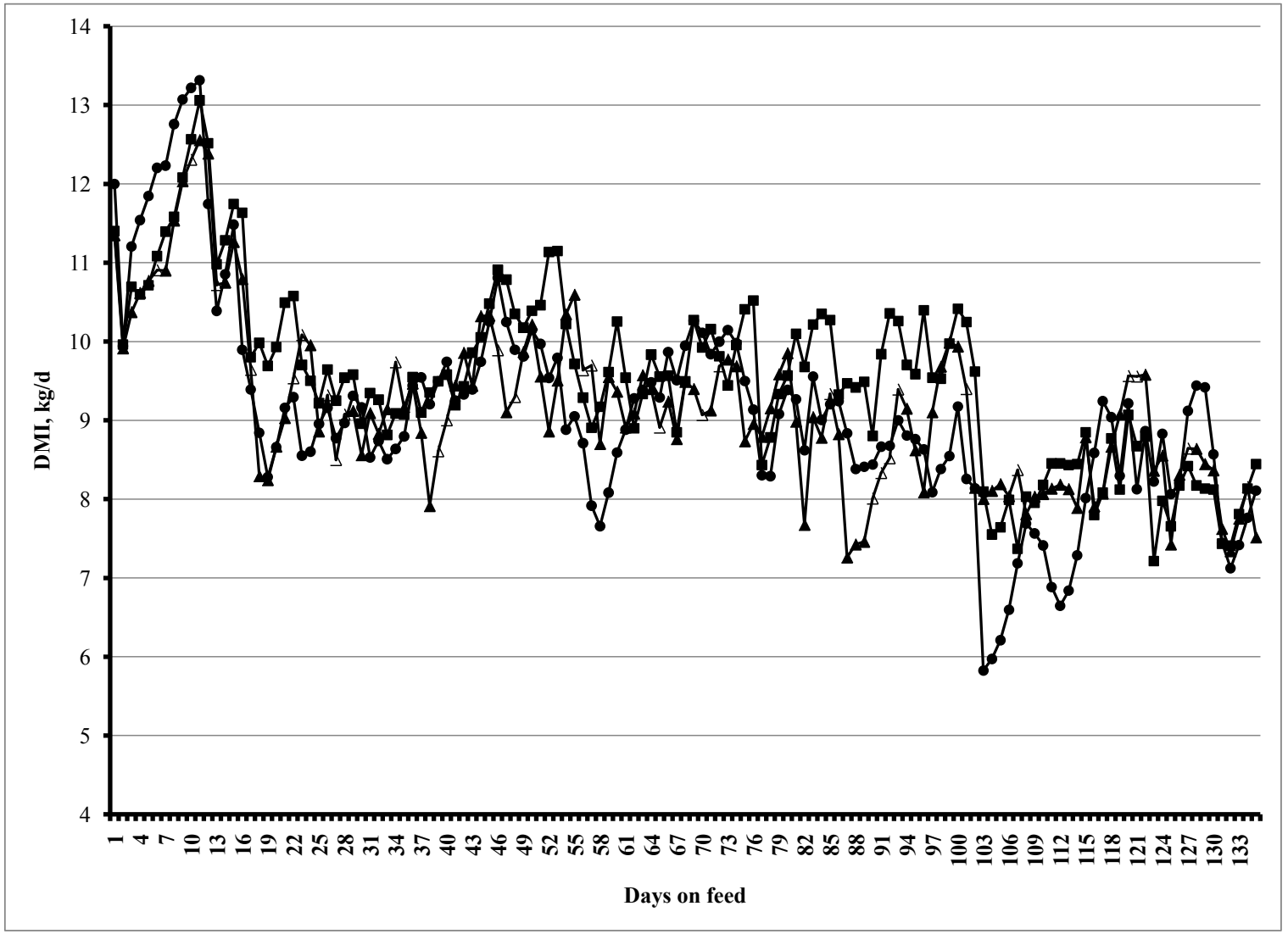
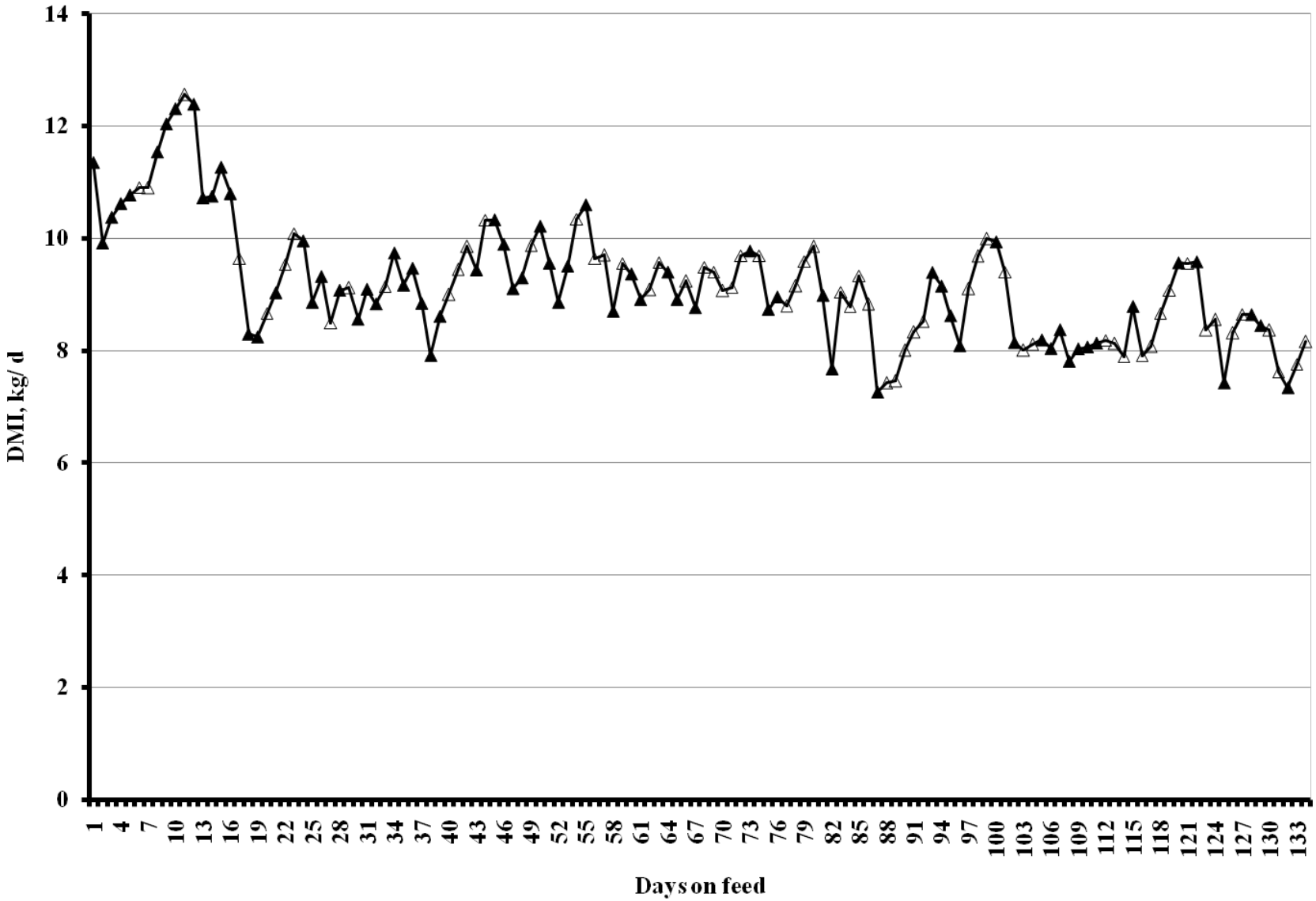

DMI, kg/d
14
13
12
11
10
9
8
7
6
5
4
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61 64 67 70 73 76 79 82 85 88 91 94 97 100 103 106 109 112 115 118 121 124 127 130 133
Days on feed

Figure 5.3 Differential effects of feeding 0.4 or 0.6 S% intermittently on feed intake by finishing steers dry-rolled corn based diets.

Open triangles indicate days on which steers were fed diets containing 0.4% S, and closed triangles indicate days on which steers were fed diets containing 0.6% S. Sulfur level had no effect on DMI ($P = 0.20$); SEM = 0.892


14
12
10
8
6
4
2
0
DMI, kg/ d
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61 64 67 70 73 76 79 82 85 88 91 94 97 100 103 106 109 112 115 118 121 124 127 130 133
Days on feed

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