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Left Running Head: Drewnoski et al.

Right Running Head: Sulfur and beef cattle

High-sulfur in beef cattle diets: a review

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ABSTRACT: While many cattle feeding areas in the United States have long dealt with high sulfate water, increased feeding of ethanol co-products such as distillers grains with solubles to beef cattle has led to a corresponding increase in dietary sulfur. As a result, sulfur metabolism in the ruminant has been the focus of many research studies over the past ten years, and advances in our knowledge have been made. Excessive sulfur in cattle diets may have implications on trace mineral absorption, dry matter intake, and overall cattle growth. This review will focus on what we have learned about the metabolism of sulfur in the ruminant, including ruminal sulfate reducing bacteria, the role of ruminally available sulfur, factors affecting the production of hydrogen sulfide in the rumen, and the potential mechanisms behind sulfur toxicity in cattle. Additionally, this review will discuss potential strategies to minimize risk of sulfur toxicity when cattle are fed high-sulfur diets, including dietary and management strategies. Further research related to high-sulfur diets including implications for carcass characteristics, meat quality, and animal health will also be discussed. As ethanol production processes continue to change, the nutrient profile of the resulting co-products will as well. Often removal of one nutrient such as oil will result in the concentration of other nutrients such as sulfur. Thus it seems even more likely that a better understanding of sulfur metabolism in the ruminant will be important to beef cattle feeding in the future.

Key words: cattle, distillers grains, hydrogen sulfide, performance, sulfur

INTRODUCTION

A tremendous amount of information concerning sulfur (**S**) metabolism in the ruminant and impacts on cattle performance has been gained in recent years, primarily fueled by an increase in the S content of cattle diets due to inclusions of high S co-products of ethanol production. Klopfenstein et al. (2008) provided a thorough review of the literature related to the effects of distillers grains (**DGS**) inclusion rate in cattle diets, irrespective of S content, on performance and carcass characteristics. However, S content is one of the major factors limiting inclusion of ethanol co-products in feedlot cattle diets. Additionally, high sulfate water continues to be problematic in many areas of the United States (Gould et al., 2002). Previous reviews on the topic of S-induced polioencephalomalacia (**S-PEM**) by Kandylis (1984) and Gould (1998) provide a thorough examination of the research available to that point. This review will attempt to summarize the wealth of new data generated on this topic. Additionally, topics in the present review will include the negative impacts of high S diets on cattle performance and carcass characteristics.

SULFUR REQUIREMENTS AND SOURCES OF SULFUR

In recent years, greater inclusion of ethanol co-products in the diets of beef cattle have resulted in increased concentration of dietary S, as the S content of DGS can range from 0.3 to 1.7%, commonly averaging 0.6-0.8% (Buckner et al., 2011; Kim et al., 2012). This range in S

content of DGS is likely due to differences across ethanol plants in their processing methods, as it is unlikely the base corn used varies this much in S. Buckner et al. (2011) reported that load to load variation of S within a plant ranged from 3 to 13%. Table 1 presents the calculated potential range of dietary S in a corn based-finishing ration when various concentrations of ethanol co-products are included in the diet, assuming within ethanol plant variation of 10%. While increasing inclusion rates of ethanol co-products may decrease ration costs for beef cattle, the risk of S toxicity also increases due to variation of S concentrations in ethanol co-products. Co-products of ethanol production continue to evolve as new technologies are developed, allowing ethanol companies to extract greater profit from their process. Currently, an estimated 85% of ethanol plants in the U.S. are extracting corn oil in some manner (K. A. Rosentrater, Distillers Grain Technology Council, Ames, IA, personal communication) resulting in a co-product with lesser oil content, which slightly increases S content because other nutrients are concentrated slightly after oil removal.

The beef cattle NRC (2000) recommends 0.15% S to support adequate growth of beef cattle. Sulfur is required for growth and metabolism of many ruminal bacteria, particularly cellulolytic bacteria (Spears et al., 1976). Additionally, S is needed as a component of the S-amino acids methionine, cysteine, and cystine, as well as the B vitamins thiamine and biotin (NRC, 2000). Cattle feedstuffs such as forage and corn primarily contain S in the form of S-amino acids. However, increased S may be introduced into the ruminant diet through a variety of sources, including high sulfate water (APHIS, 2000), molasses (NRC, 2000), or co-products of wet corn milling where sulfurous acid is utilized in the steeping process (corn gluten feed), or dry corn milling where sulfuric acid is utilized to control fermenter conditions (DGS, condensed corn distillers solubles; Kerr et al., 2008). The NRC (2005) recommends that water sulfate

concentrations should not exceed 600 mg/L for cattle. Several feedstuffs contain moderate to high concentrations of S and the reader is referred to tables from reviews by Kung (1998) and Schoonmaker and Beitz (2012) for details. Sulfur contributions from both water and feed ingredients are additive and should be used to determine total S intake by cattle. The maximum tolerable limit for S in beef cattle diets has been suggested to be 0.3% S in diets containing greater than 85% concentrate, and 0.5% in diets containing greater than 40% forage (NRC, 2005). The Mineral Tolerance of Animals (NRC, 2005) defines maximum tolerable limit as the dietary concentration that will not impair animal health or performance when fed for a set period of time. The concentration of S that limits DMI and thus decreases cattle performance appears to be less than that which causes S-PEM. For S, the maximum tolerable limit is based on prevention of poor cattle performance and increased risk of S-PEM, and not based on the avoidance of negative effects of S on absorption of critical trace minerals such as Cu or Se (NRC, 2005). The concentration of S that interferes with trace minerals is likely less than that which decreases cattle performance; however, the effect of S on trace mineral status may be more easily overcome through nutritional strategies than the negative effects of S on DMI and performance.

RUMINAL SULFATE REDUCTION AND HYDROGEN SULFIDE

Bacterial sulfate reduction may be classified as either dissimilatory, also known as sulfate respiration, or assimilatory. In assimilatory reduction, bacteria reduce sulfate to hydrogen sulfide (H_2S) which is then utilized to produce S containing amino acids or co-factors such as biotin and pantothenic acid; while in dissimilatory reduction the bacteria reduce sulfate and produce H_2S as an end product of their metabolism (Bradley et al., 2011). These dissimilatory sulfate reducing

bacteria (**SRB**) utilize anaerobic respiration pathways for their bioenergetic processes, and depend on the dissimilatory sulfite reductase enzyme activity to do so (Bradley et al., 2011). Many SRB can also reduce other oxidized inorganic S compounds (sulfite, thiosulfate, or elemental S) as well as S-containing amino acids (Coleman, 1960; Barton and Fauque, 2009). It is believed that production of H₂S in the rumen is the root cause of much of the detrimental effects of high S diets on cattle performance and health (Gould, 1998).

Sulfate reducing bacteria, which primarily use the dissimilatory pathway, are thought to compose a very small portion (less than 1%) of the ruminal bacterial population (Callaway et al., 2010), but they pose a significant threat to animal health. Unfortunately, populations of SRB in the rumen have not been well described. Scientists utilizing culture techniques have suggested thus far that the major species of SRB present in the rumen are likely members of the *Desulfovibrio* or *Desulfotomaculum* genera (Coleman, 1960; Howard and Hungate, 1976). Cummings et al. (1995a) used culture techniques to determine that several SRB species isolated from steers diagnosed with S-PEM were gram negative and similar to *Desulfovibrio* species.

Recently, genomic technology was utilized to determine the major ruminal bacterial species present when steers were fed 0.3% or 0.6% S diets (J. Koltes, J. Reecy, S. L. Hansen, unpublished data). Bacterial DNA was isolated from rumen fluid samples collected after steers received diets for 155 d (n = 6/treatment). Equal amounts of DNA were pooled by diet (n = 1 pool/diet), used to prepare libraries for sequencing on an Illumina GA IIx machine (Illumina; San Diego, CA), and metagenomic sequences were aligned to the genome using MEGAN to identify differentially represented microbial populations. In the case of either low S or high S diets, the top three genera of SRB identified were the same (*Desulfovibrio*, *Desulfohalobium*, and *Sulfolobus*); however, reads associated with *Desulfovibrio* were by far the most influenced by

dietary S (32,000 reads from the high-S pool compared with 20,000 reads from the low-S pool). Limited conclusions can be made from these data because of the lack of replication; however, clearly dietary S concentration influences ruminal bacterial population and further work is warranted in this important area.

Lewis (1953) determined that daily dosing of sulfate increased the rate of reduction of sulfate and the production of sulfide in the rumen of lambs. Alves de Oliveira et al. (1997) also reported that high dietary S resulted in a faster rate of sulfate reduction by ruminal bacteria after several weeks on a high S diet. Loerch et al. (2012) fed lambs a diet that contained 0.11% S for 8 weeks and then introduced 0, 0.2, or 0.4% S from sodium sulfate to the diet. They found it took 15 d before ruminal H₂S concentrations differed between lambs fed diets with or without supplemental S. Additionally, ruminal concentrations of H₂S continued to increase through 29 d (their final d of sampling) of exposure to added dietary S. In cattle fed a high concentrate diet containing 0.68% S, peak ruminal concentrations of H₂S were observed 14 d after consumption began, despite the fact that neither the greatest dietary S intakes or the lowest ruminal pH occurred at this time (Drewnoski et al., 2012a). Furthermore, when cattle were switched from a 0.3% S diet to a 0.6% S diet after consuming a finishing ration for 28 d, it took 14 d for ruminal H₂S to reach maximal concentrations (Drewnoski and Hansen, 2013a). Collectively, this evidence suggests that ruminal SRB may require an adaptation period in response to increased dietary S before generating maximal concentrations of sulfide. Exactly how increased dietary S leads to greater production of sulfide is unclear. Two possible mechanisms exist: 1) ruminal SRB increase in numbers in response to greater available S, or 2) SRB can increase their metabolism by utilizing a different electron donor (i.e. lactate or fumarate) or increasing the copies of dissimilatory sulfite reductase gene/SRB cell number.

Cummings et al. (1995b) reported that microbes isolated from cattle displaying symptoms of S-PEM had increased capacity to generate H₂S in ruminal fluid cultures after the animals had been fed a high-S diet for approximately 10 to 12 d. These authors also found that after being exposed to high concentrations of S the number of SRB did not appear to increase. However, based on our metagenomic analysis, the reads of *Desulfovibrio* were 1.65 fold greater in the steers fed the 0.6% S diet compared to 0.3% S diet, while *Desulfohalobium* and *Sulfolobus* were relatively similar between dietary S treatments. An increased number of reads in the samples from steers receiving 0.6% S relative to 0.3% S (1.43 to 1.65 fold greater) was found for several less abundant SRB genera, including *Desulfatibacillum*, *Desulfotomaculum*, and *Desulfobacterium*. When primers were designed to identify *Desulfovibrio desulfuricans* in these samples using real time PCR, the variation in raw copy numbers of this bacteria was highly correlated with rumen H₂S concentrations as measured 6 h post-feeding after 155 d on either 0.3% or 0.6% S diets (n = 6/diet) and accounted for 60% of the variation ($P < 0.05$) in H₂S (E. L. Richter, C. Ziemer, S. Hansen, unpublished). These data support an important role of *Desulfovibrio* in sulfate metabolism in the ruminant and suggest that certain SRB species are increased in response to a greater concentration of dietary S.

PROPOSED MECHANISMS OF SULFUR TOXICITY

Sulfur-induced Polioencephalomalacia

Polioencephalomalacia is a neurologic disease of ruminants that results in lesions of the grey matter in the brain with certain gross and microscopic features that are not specific for a

particular etiology (Gould, 1998). Polioencephalomalacia was first associated with thiamine deficiency but other toxic or metabolic diseases (e.g., acute Pb poisoning, water deprivation and high dietary S) can result in PEM. Data compiled in a recent meta-analysis by Nichols et al. (2013) suggest the incidence of S-PEM in feedlot cattle consuming diets of 0.5% S and 4% NDF from roughage is about 1%. In this study S-PEM diagnosis was based on observation of symptoms; however, the actual rate of S-PEM may be slightly higher than this as other researchers have not noted signs of PEM in the live animal but have found lesions in the brain during postmortem examination (Olkowski et al, 1992; Niles et al., 2002). Given that most of the research regarding high S diets has been conducted in small pen settings at university facilities the total number of animals receiving a high S diet in a study is generally less than 100 animals. The low incidence rate of S-PEM means that S-PEM is difficult to identify in small pen research studies.

Ruminal Production and Subsequent Inhalation of Hydrogen Sulfide

Gould (1998) suggested that S-PEM is a form of sub-acute H₂S toxicity. In experiments where S-PEM was induced by high S diets there was a positive association between S-PEM and increased ruminal H₂S (Gould et al., 1997). The accumulation of H₂S in the rumen is due to the metabolism of SRB. With a pKa of 7.04, H₂S can be converted to the HS⁻ ion in the rumen (Schoonmaker and Beitz, 2012). As pH decreases, the amount of sulfide remaining in its gaseous form will increase. The etiology of S-PEM has not yet been fully elucidated, but it is speculated that eructated H₂S gas is inhaled by the animal ultimately allowing H₂S to enter the brain, which may then cause necrosis of the grey matter (Figure 1). Dougherty and Cook (1962) observed that

70 to 80% of gas eructated from the rumen is subsequently inhaled by the ruminant, which would allow the eructated gases to enter circulation without being detoxified by the liver. Further, when Dougherty et al. (1965) infused H₂S into the rumen of sheep, they observed that sheep with an open trachea collapsed after several eructations, whereas those with a blocked trachea produced no clinical signs. These data suggest that the mechanism of toxicity is not through ruminal absorption of sulfide or H₂S, but through the inhalation of eructated H₂S. Loneragan et al. (2005) also provided compelling evidence that elevated H₂S is correlated to the incidence of S-PEM. They observed that both incidences of S-PEM and the ruminal concentration of H₂S peaked at the same time in feedlot cattle consuming high sulfate water (Figure 2).

Proposed Mechanisms of Cellular Toxicity due to Hydrogen Sulfide

The brain is an organ that has relatively high energy demand (Magistretti, 2008) and low concentration of antioxidants (Gilgun-Sherki et al., 2001). It has been thought that lesions observed in S-PEM are the result of H₂S inhibiting the electron transport chain. While H₂S has gained attention as a potentially important signaling molecule at low concentrations in the body (Li et al., 2009), excessive concentrations of H₂S can block cytochrome C oxidase, leading to depletion of ATP and ultimately causing cell death (Beauchamp et al., 1984). However, another possible mechanism by which H₂S induces cytotoxicity is by forming reactive S species and increasing the formation of reactive oxygen species thus causing oxidative damage (Truong et al., 2006). Based on this work (Truong et al., 2006), a proposed model by which H₂S may cause cellular death is shown in Figure 3. It is unclear if H₂S-induced cytotoxicity is the cause of

damage to brain cells in cattle experiencing S-PEM. Studies are needed to determine the mechanism by which brain cell death is induced in S-PEM as this may lead to potential mitigation strategies.

Concentration Threshold of Hydrogen Sulfide to Induce Polioencephalomalacia

Gould et al. (1997) suggested that S-PEM was associated with ruminal H₂S concentrations above 2,000 mg/L. In this study, 3 or the 4 steers consuming a high S diet exhibited signs of PEM. However, many studies have observed concentrations of H₂S two to three fold greater than 2,000 mg/L without observing clinical signs of PEM (Neville et al., 2010; Drewnoski et al., 2012b; Felix et al, 2011; Morine et al., 2012b; Drewnoski and Hansen, 2013a). It is likely that a difference in the time of sampling is the cause of this discrepancy. Gould et al. (1997) did not always collect samples at the same time of day, but typically took samples in the morning near the time of feeding (D. H. Gould, personal communication, 2010) whereas the studies observing significantly greater concentration of H₂S without toxicity have taken samples 4 to 9 h post-feeding. When serial H₂S samples have been taken throughout the day a diurnal pattern of H₂S was observed, thus time of sampling (relative to feeding) can have major impact on the H₂S values obtained. When cattle are fed once a day peak H₂S concentrations appear to occur between 3 to 12 h after feeding (Drewnoski et al., 2012a; Felix et al., 2011). Drewnoski et al. (2012a) observed that ruminal H₂S concentrations around 2,000 mg/L prior to feeding corresponded to concentrations of 8,000 to 9,000 at 4 to 12 h post-feeding when cattle were fed a high concentrate diet with 0.68% S. Whereas Felix et al. (2012) showed that ruminal H₂S concentrations around 2,000 mg/L prior to feeding corresponded to concentrations of 5,000 to

6,000 at 3 to 12 h post-feeding when cattle were fed a high concentrate diet containing 0.43% S. It is important to note that the diurnal H₂S pattern may be different in cattle fed twice a day rather than once a day, and it is also likely the timing of peak H₂S may be affected by the composition of the diet. Furthermore, these studies altered S consumption through manipulation of dietary S, and little information is available regarding when peak H₂S concentrations may be observed when S comes from sulfate in water.

A threshold concentration of H₂S at which S-PEM is induced remains to be determined. Niles et al. (2002) reported that all heifers receiving the diet containing 0.55 or 0.70% S exhibited clinical symptoms of PEM. The mean peak concentrations of H₂S (time of sampling not indicated) for these treatments were 14,500 and 18,642 mg/L, respectively. Loneragan et al. (2005) reported that a steer with a ruminal H₂S concentration of 13,448 mg/L succumbed to S-PEM the next day (time of sampling not indicated). In the study by Drewnoski et al. (2012a) a steer that developed S-PEM had a ruminal H₂S concentration of 12,000 mg/L, 8 h post-feeding, one day prior to the onset of symptoms. However, three other steers in the study reached or exceeded this concentration but did not show signs of PEM, suggesting that factors other than strictly H₂S concentration are critical in the etiology of S-PEM.

EFFECTS ON LIVE CATTLE PERFORMANCE

Sulfur Effects on Growth and Dry Matter Intake of Cattle

Cattle fed high-concentrate diets appear to have less tolerance for increased dietary S than cattle consuming forage-based diets. Spears et al. (2011) found DMI was not affected in

steers consuming a corn-silage based diet containing 0.12, 0.30, or 0.46% S (DM basis); however, when switched to a ground corn based-diet, DMI linearly decreased as S increased from 0.12 to 0.46% S. Tolerance for high dietary S by cattle consuming higher roughage diets likely relates to differences in ruminal pH and/or in rumen microbiome populations across these diet types.

Numerous reports have noted the negative influence of high dietary S on finishing cattle performance (Loneragan et al., 2001; Zinn et al., 1997; Uwituze et al., 2011a; Richter et al., 2012; Drewnoski and Hansen, 2013). While not as obvious as S-PEM, S toxicosis as reflected by decreased DMI and ADG is economically detrimental. The effects of increased ethanol co-product inclusion in feedlot diets on cattle performance has been well reviewed by Klopfenstein et al. (2008) and more recently by Galyean et al. (2012). Erickson et al. (2012) determined energy content of DGS (dry, modified, and wet) based on cattle ADG or G:F, and reported net energy content was from 13 to 40 percent greater than that of corn, likely due to the greater fat content of DGS. Despite a linear increase in S as DGS inclusion increases, a quadratic effect on ADG is often observed. In comparison to a corn-based finishing diet, ADG increases up to approximately 30% inclusion of wet DGS, and begins to decrease thereafter (Klopfenstein et al., 2008).

To better describe the effect of dietary S concentration on cattle performance a small meta-analysis was conducted to determine a weighted average slope for DMI, ADG and HCW as affected by S concentration of the diet (DM basis). Data for this analysis were sorted into two groups, the first being published studies which varied dietary S content strictly from inorganic S sources (S alone), avoiding potential confounding effects of altering the non-S nutrient component of a test diet (n = 4), and the second being published studies which varied S content

of the diet through altered inclusion of DGS in the diet ($n = 6$). The mixed procedure of SAS was utilized for this analysis where the model included the fixed effect of dietary S concentration, group (S alone or DGS S source) and the group \times S interaction. Study and study \times S concentration were designated as random and studies were weighted using the inverse of the variance associated with each measurement of interest (DMI, ADG, and HCW). Inclusion of the work by Zinn et al. (1997) was carefully considered; however, this work includes a very small range of dietary S (0.15-0.25% S), making extrapolation beyond these concentrations highly uncertain, and some authors (NRC, 2005) have postulated that the depression in performance by cattle in this study may have been due to the addition of ammonium to a steam flaked corn diet, rather than purely an effect of added S. Additionally, the study utilized very low concentrations of dietary S, which are unlikely to be found in present diets containing even small amounts of DGS. Therefore, it was determined that the Zinn et al. (1997) study represents a different population and it was excluded from the meta-analysis.

Interestingly, the interaction between study group (S alone or DGS) and S concentration was significant for HCW ($P = 0.02$), and tended toward significance for ADG ($P = 0.08$) and DMI ($P = 0.11$). This suggests that the effect of S on DMI, ADG, and HCW are not the same when only dietary S is increased versus when dietary S is increased as a result of greater inclusion of DGS. When inorganic sources of S, rather than increased inclusion of DGS were used to achieve greater S concentrations in finishing diets containing no ethanol co-products (Spears et al., 2011, ammonium sulfate) or moderate amounts (30 or 40% on DM basis) of co-products (Uwituze et al., 2011a, sulfuric acid; Richter et al., 2012, sodium sulfate; Pogge and Hansen, 2013, sodium sulfate), a clear detrimental effect on performance was observed. The results of this combined analysis indicate that increasing dietary S, from inorganic S sources,

beyond approximately 0.2% S negatively impacts DMI (Figure 4A), ADG (Figure 4B), and HCW (Figure 4C), with the slopes of each response being different from 0 ($P < 0.01$). Thus, with every 0.1% increase in dietary S concentration beyond approximately 0.2% S a decrease of 0.43 kg/d DMI, 0.08 kg/d ADG, and 6.6 kg HCW would be predicted.

However, when the same performance variables are plotted against dietary S concentrations from studies where dietary S was varied through increased inclusion of dried DGS (Buckner et al., 2008 and Drewnoski et al., 2013) or wet DGS (Vander Pol et al., 2006 and Corrigan et al., 2009) the effects are less pronounced. The predicted effect of S on DMI is negative, with a slope that is different from zero ($P = 0.01$; Figure 4D). Thus, increasing dietary S decreases DMI regardless of whether S is introduced from a variety of inorganic sources or through varying concentrations of DGS; however, though the effect is more pronounced when S comes from non-DGS sources. Similarly, increasing S from DGS sources negatively affects ADG ($P = 0.04$; Figure 4E); however, this predicted effect of S on ADG is less negative compared to when S is added from non-DGS sources. Conversely, the effect of S from DGS inclusion on HCW is distinctly different, as the slope in this model is not different from zero ($P = 0.73$; Figure 4F), indicating no predicted effect of S on HCW. Clearly when dietary S concentration increases due to supplementation with strictly inorganic sources, negative effects on these performance variables are noted. However, when DGS-inclusion is varied it is difficult to predict cattle performance impacts based on S concentration alone. Essentially when the energy value of DGS exceeds that of the ingredient which it is replacing (corn in these studies), it potentially masks some of the negative effects of S on cattle performance. Additionally, the feeding value of DGS in cattle diets is affected by DGS nutrient profile, type of DGS (wet, modified, dried), and interactions with other dietary components such as roughage and methods

of grain processing. Collectively, these data suggest that S concentration of DGS alone is insufficient to predict effects on cattle performance.

Few authors have directly evaluated the influence of ruminal H₂S concentrations on gain or DMI, but Uwituze et al. (2011a) reported a strong negative correlation between H₂S concentrations and both ADG (-0.58 or -0.26 for steam flaked corn and dry rolled corn, respectively) and DMI (-0.67 and -0.40 for steam flaked corn and dry rolled corn, respectively). Richter et al. (2012) noted that DMI was decreased in steers fed a 0.6% S diet, primarily during the first 29 d of the finishing diet, suggesting that cattle are particularly sensitive to high S diets during this time. The decrease in DMI coincided with a peak in H₂S concentration, suggesting that increased H₂S may cause steers to decrease feed intake, presumably because of the discomfort they experience. Increasing concentrations of dietary S may also impact rumen motility and digestibility of the diet. Uwituze et al. (2011b) reported decreased DMI in steers fed 0.65% S vs. 0.42% S regardless of corn processing method (dry rolled or steam flaked), but also reported that DM and OM digestibility were greater in steers fed the higher S diet. The authors suggested that because H₂S appears to affect smooth muscle, increased dietary S may result in decreased motility of the gastrointestinal tract, potentially causing a greater retention time of feedstuffs, and thus allowing for more complete digestion in the rumen. Interestingly, Delfiol et al. (2013) reported that sheep fed 0.9% or 1.2% S diets had less frequent rumen movements when compared with sheep fed 0.2% S diets.

Sulfur as a Trace Mineral Antagonist

In the ruminant, S is a major trace mineral antagonist. In the rumen, S can interact with molybdate to form complexes called thiomolybdates, which have very high affinity for Cu. Copper bound to thiomolybdates is unavailable for absorption and use by the animal, thus feeding high S diets causes a decrease in Cu status of the ruminant, potentially resulting in severe deficiency if not properly addressed (Suttle et al., 1991). In addition, S as sulfide, can bind to Cu and prevent absorption in the intestine (Suttle et al., 1991). Spears et al. (2011) reported that not only was liver Cu decreased in steers fed 0.31 or 0.46% S compared with 0.13% S, but also that activity of whole blood glutathione peroxidase, a Se-dependent enzyme, was lesser in 0.31% and 0.46% S-fed steers compared with 0.13% S-fed steers. In dairy cattle, Ivancic and Weiss (2001) reported that true Se digestibility was decreased as dietary S increased from 0.21% to 0.4% and 0.7%, regardless of Se concentration of the diet (0.13 or 0.27 mg Se/kg DM). Sulfur and Se share some similar chemical properties and there is evidence that the two elements compete for a common intestinal transporter (Ardüser et al., 1985). Additionally, S may compete for incorporation into selenoenzymes, thus decreasing enzyme activity (Lee et al., 2000).

Pogge et al. (2014a) found that steers receiving 0.68% S diets retained less Cu and Mn when compared with steers fed 0.20% S diets during a 5-d collection period. The maximum tolerable limit for S in cattle diets is not reflective of the concentrations of S which would negatively impact trace mineral status. Trace minerals such as Cu, Se, Mn, and Zn likely need to be included in the diet at greater than NRC recommendations when diet S concentration exceeds 0.30%. Decreased trace mineral status may contribute to decreased performance of cattle fed high S diets; however, the effects of S on trace mineral status will take some time and thus may have greater implication for ruminants fed high-S diets for long periods. In many areas of the country the cowherd is wintered on harvested forages and at least some proportion of a high S

co-product. Because of the high-forage content of these diets (greater than 40%) the risk that these females will exhibit signs of S-PEM is quite low. However, trace mineral nutrition should be considered for these animals, and increased dietary supplementation or alternate routes of supplementation, such as injection of trace minerals, may be necessary to maintain adequate growth and health of the cow and her developing fetus when dietary S exceeds 0.3%. Further research in this area is warranted.

Excessive Sulfur and Cattle Immune Function

Beyond incidence of S-PEM there are limited data available regarding the influence of high S diets on cattle health. In a meta-analysis by Nichols et al. (2012) no relationship between dietary concentration of S and incidence of observed respiratory disease or foot rot in feedlot cattle was noted. Delfiol et al. (2013) fed sheep diets containing 0.2, 0.9, or 1.2% S for a period of 111 d, and found that ruminal H₂S concentrations in sheep fed 0.9 or 1.2% S were approximately 50 fold greater than in sheep fed 0.2% S, but noted no evidence of PEM based on postmortem examination of several regions of the brain. However, the authors reported that sheep consuming 1.2% S diets had recurrent diarrhea throughout the trial and that sheep consuming 0.9% or 1.2% S diets for 111 d had greater evidence of pneumonia when lungs were examined after euthanasia.

Preliminary data by D. J. Pogge, J. Roth, and S. L. Hansen (unpublished) found that blood neutrophils isolated from non-stressed steers consuming a 0.55% S diet for 143 d had greater myeloperoxidase degranulation, indicating greater neutrophil fragility and oxidative stress, compared to neutrophils isolated from steers consuming a diet containing 0.22% S. Interestingly, the addition of vitamin C to the 0.55% S diet returned myeloperoxidase

degranulation to values that were less than the control steers, indicating an increase in neutrophil membrane strength. These data support the idea that the capacity of cattle to cope with high S diets may potentially be improved by antioxidant supplementation to the diet. Particularly in light of new receiving cattle protocols where high dietary concentrations of wet DGS are utilized as cattle transition to high grain diets, further research on the impact of increased S diets on cattle immune function is warranted.

EFFECTS ON CARCASS AND MEAT CHARACTERISTICS

Increasing S in the diet beyond approximately 0.2% S appears to negatively affect carcass characteristics of finishing cattle. As shown in Figure 4C, decreased HCW due to increasing inclusion of dietary S is a fairly consistent observation. The effects of S on other carcass characteristics have been less consistent, likely due to difference in dietary composition, cattle type and age, and timing of harvest relative to control cattle. The inclusion of ammonium sulfate to both finishing heifer and steer diets to achieve concentrations of 0.25% (Zinn et al., 1997) and 0.46% S (Spears et al., 2011) resulted in decreased LM area when compared to control cattle consuming diets less than 0.20% S (Zinn et al., 1997) or less than 0.30% S (Spears et al., 2011). No differences in marbling score were observed by Zinn et al. (1997) or Spears et al. (2011). Loneragan et al. (2001) reported that increasing water sulfate concentration from 136 to 2,360 mg/L (equivalent to 0.18 to 0.40% S as a percent of the diet DM) decreased HCW, dressing percentage, and yield grade. These authors also reported a quadratic effect of sulfate on backfat, with greatest backfat noted in steers consuming 600 mg sulfate/L drinking water, and no difference in marbling scores. In most studies, when dietary S did not impact backfat, marbling

scores were not impacted either (Zinn et al., 1997; Uwituze et al., 2011a; Richter et al., 2012). One factor that may need greater consideration is the number of days cattle are fed, as cattle finished on high S diets may require additional days on feed to reach a similar body composition because of the noted negative impacts of S on DMI, ADG, and therefore, HCW.

While the effects of differing inclusion rates of DGS on aspects such as shelf life and tenderness of beef products have been evaluated (Depenbusch et al., 2009; Kroger et al., 2010), few reports on the effect of the S concentration of the diet on meat quality are available.

High S in the body has been identified as a potential cause in the development of oxidative stress, as evident by a depletion of glutathione (**GSH**; Truong et al, 2006; Pogge and Hansen, 2013), which may contribute to an oxidative environment postmortem. It is well established that a postmortem oxidative environment interferes with proteolysis thus hindering the tenderization process (Guttmann and Johnson, 1998; Lametsch et al., 2008; Rowe et al., 2004). Pogge et al. (2014b) recently reported feedlot diets containing 0.55% S (40% dried DGS diets, with sodium sulfate providing additional S) negatively altered the autolysis of the muscle protease μ -calpain, reflected by a greater percentage of the 80-kDa subunit (+9.4%) and a lesser quantity of the 76-kDa subunit (-10.5%) when compared to steers consuming diets containing 0.22% or 0.34% S. These data indicate that less autolysis was occurring in the high S diet, which in turn may explain the observed tendency for a lesser extent of troponin T degradation within the high S treatment. Pogge et al. (2014b) suggest that diets exceeding 0.34% S may result in negative impacts on the tenderness and eating quality of beef. Due to the variability in content and animal response to S, more research is needed to determine the impacts of dietary S on meat quality.

FACTORS AFFECTING HYDROGEN SULFIDE PRODUCTION

Ruminally Available Sulfur

The production of H₂S in the rumen is dependent on the availability of sulfate for reduction by ruminal SRB. Recently, Sarturi et al. (2013a) proposed the adjusted ruminal protein S (ARPS) concept when considering the potential for dietary S to result in excess H₂S production. They argue that S must be ruminally available (soluble) for reduction by SRB in order to potentially contribute to H₂S production in the rumen. Organic sources of S such as S-amino acids in corn (and thus concentrated in DGS) should be less available for ruminal reduction by SRB because at least some proportion of the dietary protein will be ruminally undegradable, preventing interaction between SRB and S-amino acids. Alternately, the excess S in DGS which comes from the use of sulfuric acid during processing should be completely available to SRB for reduction to sulfide, thus heavily contributing to ruminal H₂S production. Similarly, other sources of inorganic S such as calcium sulfate or ammonium sulfate would also be expected to be 100% available for reduction by SRB. Nichols et al. (2013) reported an average total S content and calculated ARPS content of several common feedstuffs utilized in feedlot cattle nutrition. For example, these authors reported that while total S content of wet DGS was 0.79%, they estimated ruminally available S was 0.56% (71% of the total S), when the total S content of condensed corn distillers solubles was 1.12% S, the estimated ruminally available S was 1.08% S (96% of the total S) and when steam flaked corn contained 0.14% S, an estimated 0.06% was ruminally available S (43% of the total S). Indeed when the ARPS theory was examined with different sources of S and feeds, Sarturi et al. (2013a) reported that ARPS

intake ($\text{g}\cdot\text{steer}^{-1}\cdot\text{d}^{-1}$) accounted for more of the variation in ruminal H_2S concentrations than total S intake (58 vs. 29%, respectively).

Brasche et al. (2012) tested the concentration of rumen H_2S when fistulated steers were fed one of five sources of dietary S in a Latin square design: 40% dry DGS, or additional S from: sulfuric acid, calcium sulfate, sodium sulfate, or condensed corn distillers solubles. Non-DGS S sources were added to the diet to contribute a comparable amount of S as that expected to be in the 40% DGS diet as that expected to come from sulfuric acid in the DGS (as a byproduct of the ethanol production process). When S from these different sources was balanced in this manner it was found that ruminal H_2S concentrations did not differ across treatments, supporting the idea that S from sulfuric acid in dry DGS has similar availability and potential for H_2S production as did S from inorganic sources, such as sodium sulfate. However, factors other than ruminally available S also affect H_2S production in ruminants.

Roughage Neutral Detergent Fiber Concentration

Recently, Nichols et al. (2013) conducted a meta-analysis of finishing studies conducted at the University of Nebraska feedlots over a 7 yr period and suggested that there is a strong effect of the concentration of roughage NDF within a concentration of dietary RAS on the incidence of S-PEM. Risk of S-PEM decreased approximately 19% for each 1% increase in roughage NDF in the diet, within a given concentration of RAS.

Decreased ruminal pH of cattle that consume high concentrate diets may partially contribute to increased S toxicity by increasing the amount of sulfide that remains as H_2S . There is also evidence that the uptake of sulfate by SRB may be in symport with H^+ (Cypionka, 1989),

indicating that sulfate uptake and subsequent production of H₂S would likely increase at the lower pH associated with concentrate diet-feeding of ruminants.

Morine et al. (2014) tested two different roughage sources, chopped corn stalks and chopped bromegrass hay, at three concentrations of roughage NDF (4, 7, or 10% added NDF from roughage) using a Latin square design with cannulated steers fed DDGS-based diets (0.45% S). The authors observed that ruminal H₂S was lesser in steers fed 7 or 10% roughage NDF, compared with those consuming 4% roughage NDF, regardless of roughage source. Rumen pH taken immediately after H₂S measures was found to be strongly negatively correlated with rumen H₂S concentrations, until rumen pH exceeded 5.8, above which rumen pH was a poor predictor of H₂S concentrations.

In a follow up study, Morine et al. (2012b) utilized steers fed 0.46% S diets containing 3.5, 5.7, 7.9, 10.1, or 11.4% added NDF from chopped bromegrass hay through an 84-d finishing trial. Ruminal H₂S concentrations measured over the course of the trial were linearly decreased as roughage NDF increased, ranging from 7,365 mg/L (1,756 SE) in 3.5% roughage NDF diets, to 4,528 mg/L (1,563 SE) in 11.4% roughage NDF diets. Ruminal pH, measured via rumenocentesis, again had a strong negative linear correlation with ruminal H₂S concentrations. While in many studies ruminal pH is well correlated with H₂S concentrations of ruminants fed high S diets, pH does not explain all of the variation in H₂S measures. Additionally, while H₂S concentrations were lessened in this study, adding roughage NDF to high S diets did not impact ADG, but did linearly increase DMI and weakly tended to linearly ($P = 0.12$) decrease feed efficiency (Morine et al., 2012b). Similarly, Huber et al. (2012) fed finishing diets containing 0.28 or 0.56% S to steers consuming 5, 10, or 15% grass hay (DM basis) and found that while increasing roughage increased DMI and decreased feed efficiency, no interaction between S and

roughage inclusion on steer live performance, HCW, backfat, REA, or marbling score was observed. Huber et al. (2012) did not determine ruminal H₂S concentrations of steers during their study. Morrow et al. (2013) investigated the addition of 7 or 14% long-stemmed hay (DM basis) to diets containing high S (60% dry DGS; 0.43% S in total diet) or low S (corn-based, 0.13% S). These authors also reported no effect of increasing roughage in the high S diet on cattle performance, but within the high S diets, a negative correlation between ruminal pH and ruminal H₂S measured over a 12 h period post-feeding was apparent. Collectively, these data suggest that while increasing roughage content of finishing diets containing increased concentrations of DGS (which are often high in S) likely decreases H₂S concentrations and the potential for S-PEM, it does not appear to benefit cattle performance.

It is unclear if the decrease in ruminal H₂S due to increasing effective NDF is strictly due to increased ruminal pH or whether shifts in microbial ecology of the rumen or changes in eating behavior may also be contributing factors. Increasing effective NDF in a high concentrate, high S diet, may have decreased H₂S and increased pH in part due to increasing chewing and saliva production. Shain et al. (1999) observed that time spent eating and rumination were increased in steers supplemented with 4.85% NDF from alfalfa, wheat straw or corn cobs compared with steers fed an all concentrate diet (dry rolled-corn). Additionally, these authors identified a positive relationship ($r = 0.80$) between chewing time (eating and rumination events) and ruminal pH. In the study by Morine et al. (2014) where steers were fed diets containing 0.45% S and 4, 7, or 10% NDF from bromegrass hay or cornstalks it was noted that the time spent eating at the bunk increased with increased NDF, and that rate of DMI slowed with greater NDF concentrations. More work is necessary to clarify this complex relationship between ruminal pH and H₂S as not all authors have noted a significant correlation between ruminal pH and ruminal

H₂S concentrations (Sarturi et al., 2013b), which may be due to differences in H₂S measurement methodology and sensitivity.

Adaptation to Finishing Diets

Multiple studies have suggested that H₂S concentrations increase during adaptation to a finishing diet containing increased concentrations of dietary S, with a peak in H₂S production reached somewhere between d 14 and 60 of the finishing period. Loneragan et al. (2005) utilized three concentrations of sulfate in the drinking water (136, 583, 2,360 mg sulfate/L) of feedlot cattle and observed that ruminal H₂S concentrations peaked on d 31 of the study, after which concentrations decreased and stabilized for the remainder of the trial. Drewnoski et al. (2012b) found that cattle consuming a high S diet had concentrations of H₂S that increased relative to their S intake during the first 30 d on a finishing diet, regardless of whether or not cattle were previously exposed to increased dietary S while on a high roughage diet. Delfiol et al. (2013) recently reported that in sheep fed 0.9 or 1.2% S (in diets containing 31% oat hulls and 5% bermudagrass hay) rumen H₂S concentrations reached a peak approximately 5 to 7 wk into the finishing period. When steers were fed a high S finishing diet, peak H₂S concentrations were observed from d 7 through 28 of the finishing period and this peak did not appear to be related to ruminal pH, as ruminal pH did not differ across the finishing period (Drewnoski and Hansen 2013a).

In their recent longitudinal analysis, Nichols et al. (2013) reported that of the 27 cases of PEM in feedlot cattle noted across the experiments, approximately half occurred during the first

60 d on feed. These data suggest that cattle adapting to finishing diets are at a greater risk for increased concentrations of H₂S and possibly the development of S-PEM.

During the adaptation to a high concentrate diet, changes occur in bacteria and protozoa populations. However, it is unknown how SRB are affected during this change. The SRB that have been isolated from the rumen of sheep appear to be able to use lactate as a carbon source but not acetate, propionate, or butyrate (Coleman, 1960; Howard and Hungate, 1976), so it is possible that SRB populations and/or their metabolism of sulfate increase during adaptation to high concentrate rations due to the increased availability of lactate. Initially, SRB may be able to take advantage of the increased availability of lactate, but may have to compete with rumen microorganisms such as *Megasphaera elsdenii* for lactate later in the finishing period, thus decreasing their H₂S production. Future research should attempt to profile the SRB and other microbial populations throughout the early finishing period to determine if species shifts or population increases may explain the apparent lag time for peak H₂S concentrations in cattle fed moderate to high S diets.

Steam Flaked Corn versus Dry Rolled Corn

Klopfenstein et al. (2008) and Gaylean et al. (2012) have thoroughly reviewed the literature related to potential interactions among grain processing and DGS inclusion rate in feedlot cattle diets on cattle performance, finding minimal interactions when dietary fat was accounted for. However, few studies have focused on the interaction between dietary S concentration and grain processing method. Neville et al. (2012) reported that H₂S concentrations were not influenced by corn processing method when dry DGS were fed at 20, 40

or 60% of the diet. Similarly, Uwituze et al. (2011a) reported no interaction between dietary S content (30% dry DGS in the diet; 0.42 or 0.65% total diet S, with extra S added from sulfuric acid) and corn processing method for ruminal H₂S concentrations. Neither May et al. (2009) or Uwituze et al. (2011b) noted any interaction between DDGS concentration (0 or 25% dry DGS; May et al., 2009) or dietary S concentration (0.42 or 0.65% S; Uwituze et al., 2011b) and corn processing method on digestibility of DM, OM, NDF, starch, or ether extract. When DGS is used as a main ingredient in the diet the performance difference between having steam flaked corn and dry rolled corn may not matter in terms of S metabolism, but rather may be due to changes in digestion of the feeds and not changes in SRB metabolism.

ADDITIVES TO MANAGE HIGH-SULFUR DIETS

Thiamine

At one time it was thought that S-PEM was due to thiamine deficiency caused by ruminal destruction of thiamine (Goetsch and Owens, 1987; Gooneratne et al., 1989). However, when a semi-synthetic thiamine-free diet was fed to weaned lambs to test the effect of high S on the microbial production of thiamine, neither rumen thiamine concentration or thiaminase activity were modified by the dietary S content (0.2 vs. 0.6% S; Alves de Oliveira et al., 1996). Although Alves de Oliveira et al. (1997) reported that high S slightly decreased microbial thiamin production (326 vs. 266 nmol/d) *in vitro*, they concluded that it was unlikely to result in a substantial effect on thiamine status of the animal. In his review on S-PEM, Gould (1998) summarized data that suggests experimentally induced S-PEM (Sager et al., 1990; Gould et al.,

1991) as well as some field cases of S-PEM (Mella et al., 1976) were not caused by thiamine deficiency. This is not the only evidence that high dietary S can induce PEM independent of a systemic or local thiamine deficiency. Olkowski et al. (1992) found that sheep fed a 0.63% S diet had slightly increased liver and blood thiamine concentrations compared to sheep fed a 0.19% S diet and that brain thiamine concentrations were unaffected by S concentration. Furthermore, baseline activity of the thiamine-dependent enzyme erythrocyte transketolase, and erythrocyte transketolase activity measured after addition of thiamine pyrophosphate (often used as a diagnostic marker of thiamine deficiency), were not affected by dietary S concentration in this study (Olkowski et al., 1992). In addition, feedlot cattle consuming high sulfate water (2,200 and 2,800 mg sulfate/L) exhibiting acute signs of PEM were found to have blood thiamine concentrations within normal reference ranges (McAllister et al., 1997). Likewise, Loneragan et al. (2005) reported that blood thiamine concentration was not affected by consumption of water with increased concentrations of sulfate (136, 583, or 2,360 mg sulfate/L water).

So why do many nutritionists feed supplemental thiamine in situations of high dietary S consumption? This may be because thiamine has nonspecific therapeutic benefits in cerebral diseases (Gould, 1998). Thiamine injections are the primary method of treatment for animals afflicted with PEM, regardless of cause (Ensley, 2011). Indeed PEM induced by acute Pb poisoning has been shown to be thiamine responsive (Gould, 1998). Thiamine plays a key role in the tri-carboxylic acid cycle and pentose shunt and thus may increase energy availability to the diseased brain. Additionally, thiamine may reduce edema in the brain through thiamine-dependent enzymes (Olkowski et al., 1992). Thiamine supplementation (229 mg/kg of diet) has been shown to prevent clinical signs of S-PEM in lambs (Olkowski et al., 1992). However, it did not prevent the manifestation of PEM as determined by the presence of brain lesions at slaughter

(Olkowski et al., 1992). Thus thiamine supplementation may be seen as a safeguard against clinical signs but does not completely protect the brain from damage.

Few studies have evaluated the potential performance benefits of supplementing thiamine in situations of high S consumption. When thiamine was supplemented at a rate of 1 g/d in the feed of growing cattle consuming high S water (3,786 mg sulfate/L) ADG was increased from 0.49 to 0.63 kg/d (SEM \pm 0.04; Ward and Patterson, 2004). More recently, Neville et al. (2010) tested the effectiveness of four concentrations of dietary thiamine (0, 50, 100, or 150 mg supplemental thiamine/d) in lambs fed 0.73% S, DDGS-based diets, through a 110 d finishing period. In their first experiment, there was a quadratic ADG response to thiamine supplementation with the lambs receiving 50 mg of thiamine having a slightly increased ADG compared to the control (0.274 vs. 0.268 kg/d; SEM of 0.005). In their second experiment no response to thiamine in ADG was observed. In both experiments, no evidence of PEM was noted when brains were examined after slaughter. Further studies are needed to draw firm conclusions about the judicious use of supplemental thiamine for ruminants consuming high S diets.

Antioxidants

Exposure to high concentrations of S, and therefore H₂S, has been identified as a potential culprit in the generation of oxidative stress both *in vitro* and *in vivo* (Truong et al., 2006; Pogge and Hansen, 2013; Figure 3). In these studies, S-induced oxidative stress was noted by a decrease in reduced GSH relative to its oxidized form (**GSSG**) in cultured rat hepatocytes (Truong et al., 2006) or beef liver collected after steers received 0.55% S diets for 143 d (Pogge and Hansen, 2013). In both studies, the depletion of GSH by S suggests the importance of GSH

in detoxification of excess S. Depleting GSH may increase strain on other body antioxidants such as vitamin C, vitamin E, and trace mineral-dependent enzymes such as superoxide dismutase, catalase, and glutathione peroxidase; which, under normal conditions are able to neutralize radical species through the donation of electrons. As noted earlier, the absorption of some trace minerals necessary for the catalytic activity of many antioxidants is lessened when cattle consume high S diets, potentially increasing the likelihood of oxidative stress in these animals. While it is well established that S negatively affects trace mineral status (Suttle, 1991), no research addresses the repercussions of high S diets on the integrity of the trace mineral-dependent antioxidant enzymes. Pogge and Hansen (2013) noted that supplementation of 10 g vitamin C·steer⁻¹·d⁻¹ significantly decreased the amount of GSSG in liver. Because vitamin C and GSH share a regenerative relationship, indicating one may regenerate the reduced form of the other, the results of Pogge and Hansen (2013) may imply that supplementation of an antioxidant to a high S diet could aid in prevention of oxidative stress by replenishing GSH stores (Figure 3). However, while supplementation with antioxidants may decrease oxidative stress associated with high S diets, more research is needed to determine if cattle growth or carcass characteristics may be positively affected as well.

Ionophores

Kung et al. (2000) tested the effects of lasalocid and monensin (5 mg/L in culture fluid) on H₂S production *in vitro*, and observed no effect of lasalocid but found that monensin increased the production of H₂S. The culture fluid in this experiment contained 136 mg of S per L. However, when Quinn et al. (2009) tested the effects of monensin and lasalocid (5 mg/L in

culture fluid) *in vitro*, no effects on H₂S production were noted. In their study the culture fluid of the high S treatment contained 1.75 mg of S per L. Additionally, Smith et al. (2010) observed no effects of monensin (2, 4, or 6 mg/L of culture fluid) on H₂S production when S was added to the culture fluid at 0.75, 1.50 or 3.0 mg of S per L. If one assumes that a 430 kg feedlot steer has 47 L of rumen fluid volume and intake of 10.8 kg DM/d (Clary et al., 1993), then the addition of 5 mg ionophore·L of rumen fluid⁻¹·d⁻¹ would be achieved if the diet contained 22 mg ionophore/kg DM, equating to 235 mg ionophore·steer⁻¹·d⁻¹. Thus the concentrations of ionophores tested in these studies are likely similar to what would occur *in vivo*. If the diet of the feedlot steer contained 0.5% S then the S concentration in the rumen fluid would be approximately 1.1 mg S per L, assuming no loss to H₂S or absorption of sulfide. Thus the concentrations of S used by Quinn et al. (2009) and Smith et al. (2010) are more likely to be encountered *in vivo* than that of Kung et al. (2000). *In vivo*, Felix and Loerch (2011) found that when monensin was added at 33 mg/kg DM to diets of finishing cattle containing 0.5% S, a tendency for a decrease in ruminal H₂S was observed. However, in later *in vivo* experiments, no effects of monensin (22, 33, or 44 mg monensin/kg of diet DM) were observed in cannulated steers fed diets containing 0.45% S (Felix et al., 2011). Although the effect of ionophores on H₂S has been variable, it appears unlikely that ionophores have a substantial effect on H₂S production.

Other Compounds

Research regarding mitigation of the toxic effects of high S in ruminant diets has centered around three basic strategies: inhibiting the metabolism of SRB, binding sulfide in the rumen fluid, or increasing ruminal pH in order to decrease the amount of sulfide that remains as H₂S.

Kung et al. (1998) found that adding 10 mg/L of 9, 10 anthraquinone to *in vitro* culture fluid decreased sulfide production by 71%. Regrettably, the use of this compound is likely limited by its availability and cost. Others have evaluated the use of clay minerals such as zeolite (Knight et al., 2008) or clinoptilolite (Cammack et al., 2010) as hydrogen sinks to lessen H₂S production in cattle fed high S diets, but in both cases, the clay mineral had no effects on cattle performance.

Molybdate has been shown to inhibit SRB in many environments, including rumen fluid (Gawthorne and Nader, 1976; Kung et al., 2000). Kung et al. (2000) showed that the addition of 10 mg/L of Mo to *in vitro* culture fluid decreased H₂S by 11% and 25 mg Mo/L decreased H₂S by 77%. Unfortunately, Mo can bind to Cu in the rumen causing the formation of an insoluble complex, thereby decreasing the availability of Cu to the animal, and causing potential for Cu deficiency. To achieve concentrations of 10 mg Mo/L of rumen fluid the Mo concentration in the diet would need to be at least four times the maximum tolerable level of 10 mg of Mo/kg of diet DM, which was determined based on the deleterious effects of Mo on Cu metabolism in the ruminant (NRC, 2005). Kessler et al. (2012) reported that supplementing Mo at a rate of 187.5 mg/kg DM to steers consuming high sulfate water (2,218 mg sulfate/L; ~0.073% S) substantially decreased liver Cu concentrations, and unfortunately, resulted in greater ruminal H₂S concentrations than steers receiving the high sulfate water alone.

Copper has the potential to bind with sulfide in the rumen. Felix et al. (2012) added Cu at 100 or 200 mg/kg to diets containing 60% DDGS to test if ruminal H₂S concentrations would be decreased. They observed an inconsistent response, as supplementing 100 mg Cu/kg of DM resulted in a decrease in H₂S concentration, whereas supplementing 200 mg Cu/kg DM did not result in any difference from the control treatment. The maximum tolerable level of Cu in beef

cattle diets is reported as 100 mg/kg of diet DM (NRC, 2000). The concentrations of Cu in the liver of these finishing cattle after 168 d on diet were 86, 708, and 933 mg/kg (DM) for the control, 100, and 200 mg/kg Cu, respectively. This suggests that feeding these high concentrations of Cu could result in liver damage. The source of Cu (basic copper chloride) used in the study by Felix et al. (2012) is not highly soluble in the rumen environment (Spears, 2003; Genter and Hansen, 2013), which may have decreased the availability of Cu to interact with sulfide in the rumen. Use of a highly ruminally soluble source of Cu, available for binding to sulfide, may have resulted in less Cu accumulation in the liver and potentially decreased H₂S.

Some researchers have tested manganese oxide (Mn(II)O) as a potential mitigator of ruminal H₂S production (Kelzer et al., 2010). When the effects of adding various concentrations of Mn(II)O to *in vitro* fluid was tested, the results were inconclusive as H₂S production responses were inconsistent (Kelzer et al., 2010). When 1000 mg Mn/kg from Mn(II)O was added to the diets of cannulated steers consuming a 0.65% S feedlot diet ruminal pH was increased and H₂S concentration was decreased by 30% at 6 hr post feeding in comparison to when these steers were fed the diet without supplemental Mn(II)O (Kelzer et al., 2012). Finishing cattle fed diets containing 0.35 or 0.6% S with 1000 mg of Mn/kg from Mn(II)O tended to have increased ADG during the first 28 d on feed but ADG was not affected during the rest of the feeding period (Kelzer et al., 2012). Supplementation of Mn(II)O did not affect DMI in this study. Ruminal H₂S was not measured, but the increased performance during the first 28 d of finishing may be due to Mn(II)O decreasing H₂S during the time in which peak concentrations of H₂S have been observed by others.

Sulfate reducing bacteria, most prominently *Desulfovibrio*, are also referred to as dissimilatory metal reducing bacteria because of their capability to reduce heavy metals (at

certain states) such as Au, Cr, Fe, Mn, Mo, and Se, to name a few (Barton and Fauque, 2009). Recently, inclusion of ferric Fe was evaluated as a method of decreasing H₂S concentration (Drewnoski et al., 2013; Drewnoski and Hansen, 2013b; Drewnoski et al., 2014). It was theorized that dissimilatory ferric Fe reduction could inhibit sulfate reduction and that the resulting ferrous Fe could also bind with some of the sulfide in the rumen fluid. Since ferric Fe has a greater redox potential than sulfate, adding a soluble form of ferric Fe could decrease ruminal sulfate reduction by competing for the same electron donors. Furthermore, some species of *Desulfovibrio* can carry out ferric Fe reduction, thus these SRB could potentially transfer their activity from sulfate reduction to Fe reduction. When the effect of ferric citrate and ferric ammonium citrate on *in vitro* production of H₂S was tested, it was found that inclusion of either source of ferric Fe at 71 mg/L resulted in a 51% reduction of H₂S. However, at concentrations of Fe in the fluid above 71 mg/L, ferric ammonium citrate was less effective than ferric citrate (Drewnoski et al., 2014). To achieve addition of 71 mg of Fe/L of rumen fluid inclusion of 290 mg of Fe/kg of diet DM would be needed. Follow up *in vivo* experiments observed a linear decrease in concentration of ruminal H₂S when four concentrations of Fe (0, 200, 300 or 400 mg/kg diet DM) from ferric ammonium citrate were included in a 0.46% S diet, using 8 steers in a repeated Latin square design. Additionally, 300 mg Fe/kg DM from ferric ammonium citrate added to a 0.5% S diet did not impact apparent absorption or liver concentrations of Cu, Mn, or Zn in steers in a follow-up digestibility study. However, when ferric ammonium citrate (300 mg Fe/kg diet DM) was fed to feedlot cattle receiving varying inclusion rates of DDGS in a larger scale study, no effects on ADG, DMI, or H₂S concentrations were observed (Drewnoski et al., 2013). The lack of effect of ferric ammonium citrate on ruminal H₂S observed in this larger scale experiment may be due to the light sensitive nature of this source of ferric Fe as it can be reduced

to its ferrous form by ultraviolet light. Ferrous Fe cannot competitively inhibit sulfate reduction and is substantially less effective at decreasing H₂S (M. Drewnoski and S. Hansen, unpublished data). Future studies on the effectiveness of ferric Fe are warranted, but a more stable source of soluble Fe such as ferric citrate should be utilized.

CONCLUSIONS

High S cattle diets are generally a result of high sulfate water or inclusion of ethanol co-products. As long as ethanol co-products remain competitively priced relative to corn, and their nutritional value remains equal or greater to that of corn, high S cattle diets may remain inevitable for some producers. Currently, no magic bullet exists in the battle against S toxicity. However, sound cattle management and an understanding of ruminal S metabolism and how dietary factors affect H₂S are the best weapons against S toxicity. To date, the most valuable tools for a nutritionist or cattle feeder in the prevention of S-induced toxicity appear to be a strong understanding of the ruminal availability of the S in the diet and inclusion of sufficient dietary roughage. A minimum of 7 to 8% NDF from a roughage source should be included in diets containing 0.4% or more S, to minimize risk of S-PEM. Alternative means to cope with the negative effects of S on cattle health and performance need to be identified. Future research in this area should focus on identifying additional factors which affect H₂S production by SRB in the rumen of cattle fed high S diets as targets for mitigation strategies. Since H₂S cytotoxicity may be because of oxidative damage, and the trace mineral status of cattle fed high S diets may be compromised due to ruminal antagonisms, future research should focus on antioxidants as potential ameliorators of S-toxicity.

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Table 1. Range of dietary sulfur (S)¹ in corn-based finishing rations assuming 10% load to load variation in sulfur concentration of ethanol co-products

	Co-product inclusion, % of diet				
	20	30	40	50	60
% S expected in co-product	Dietary S, %				
0.3	0.16-0.17	0.18-0.18	0.20-0.21	0.22-0.23	0.23-0.25
0.4	0.18-0.19	0.21-0.22	0.24-0.25	0.27-0.29	0.29-0.32
0.5	0.20-0.21	0.24-0.27	0.28-0.30	0.32-0.34	0.35-0.38
0.6	0.22-0.24	0.26-0.30	0.32-0.34	0.37-0.40	0.41-0.45
0.7	0.24-0.26	0.28-0.33	0.36-0.39	0.42-0.45	0.47-0.51
0.8	0.26-0.28	0.33-0.35	0.40-0.43	0.52-0.56	0.53-0.58
0.9	0.28-0.30	0.36-0.38	0.44-0.47	0.52-0.56	0.59-0.65
1.0	0.30-0.32	0.39-0.41	0.48-0.52	0.57-0.62	0.65-0.71

¹Assumes no sulfur coming from drinking water and that non co-product feedstuffs contain 0.13% S.

Figure Captions

Figure 1. The ruminal metabolism of sulfate by sulfur reducing bacteria (**SRB**) and the proposed pathway of sulfur toxicity in ruminants. Excess ruminally available sulfur is reduced by dissimilatory SRB to hydrogen sulfide (**H₂S**) and excreted into the ruminal fluid. In a pH-dependent manner, some of the H₂S will dissociate to hydrosulfide ion (**HS⁻**) and remain in the fluid and the remaining H₂S will migrate to the gas cap in the rumen. Based on the pKa, at pH 7 in the fluid 50% of H₂S will dissociate to HS⁻ and at a pH 5.5 only 5% will dissociate to HS⁻. The accumulated H₂S is eructated and subsequently inhaled. This inhaled cytotoxic H₂S enters the blood stream and can cause brain lesions.

Figure 2. Example relationship between ruminal hydrogen sulfide concentrations and frequency of S-induced polioencephalomalacia (**S-PEM**) in steers consuming high sulfate water (used with permission, originally published by Loneragan et al., 2005 in *The Bovine Practitioner*).

Figure 3. The proposed mechanism by which hydrogen sulfide (**H₂S**) contributes to cytotoxicity and cellular death, and how trace minerals and vitamins may contribute to alleviating the negative effects imposed by H₂S. Hydrogen sulfide can inhibit cytochrome *c* oxidase of the electron transport chain (**ETC**), contributing to the production of superoxide ions (**O₂[•]**) by combination of O₂ with leaked electrons (**e⁻**) of the ETC. Hydrogen sulfide can also contribute to the release of cellular Fe (Fe²⁺ and Fe³⁺) from the Fe storage protein ferritin, contributing to reactive sulfur species (**RSS**) and hydroxyl radical (**OH[•]**) formation via the Fenton reaction (Truong et al., 2006). The activity of Mn superoxide dismutase (**Mn-SOD**) and Cu-Zn superoxide dismutase (**Cu-Zn SOD**) converts the O₂[•] to a less damaging compound, hydrogen

peroxide (H_2O_2). Hydrogen peroxide is converted to water via glutathione (**GSH**), Se-dependent glutathione peroxidase (**GSH-Px**), catalase, or vitamins C and E.

Figure 4. Effect of increasing dietary S from strictly inorganic sources (panels A, B, and C) or from inclusion of distillers grains (panels D, E, and F) on DMI, ADG, and HCW of feedlot cattle. Data set for panels A, B, and C included treatment means from Spears et al. (2011), Uwituze et al. (2011a), Richter et al. (2012), and Pogge and Hansen (2013). Data set for panels D, E, and F included treatment means from Vander Pol et al. (2006), Buckner et al. (2008), Corrigan et al. (2009), and Drewnoski et al. (2013). Data depicted are means from each study with the dashed line (...) indicating the predicted slope based on the meta-analysis. Probability values that the predicted slope is different from zero: A) $P = 0.01$; B) $P = 0.01$; C) $P = 0.01$; D) $P = 0.01$; E) $P = 0.04$; and F) $P = 0.73$.

Figure 1.

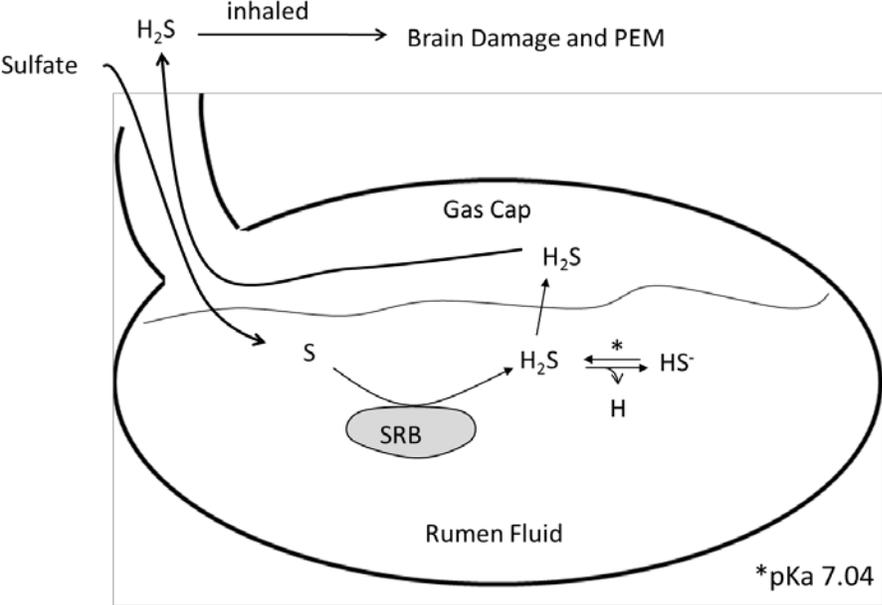


Figure 2.

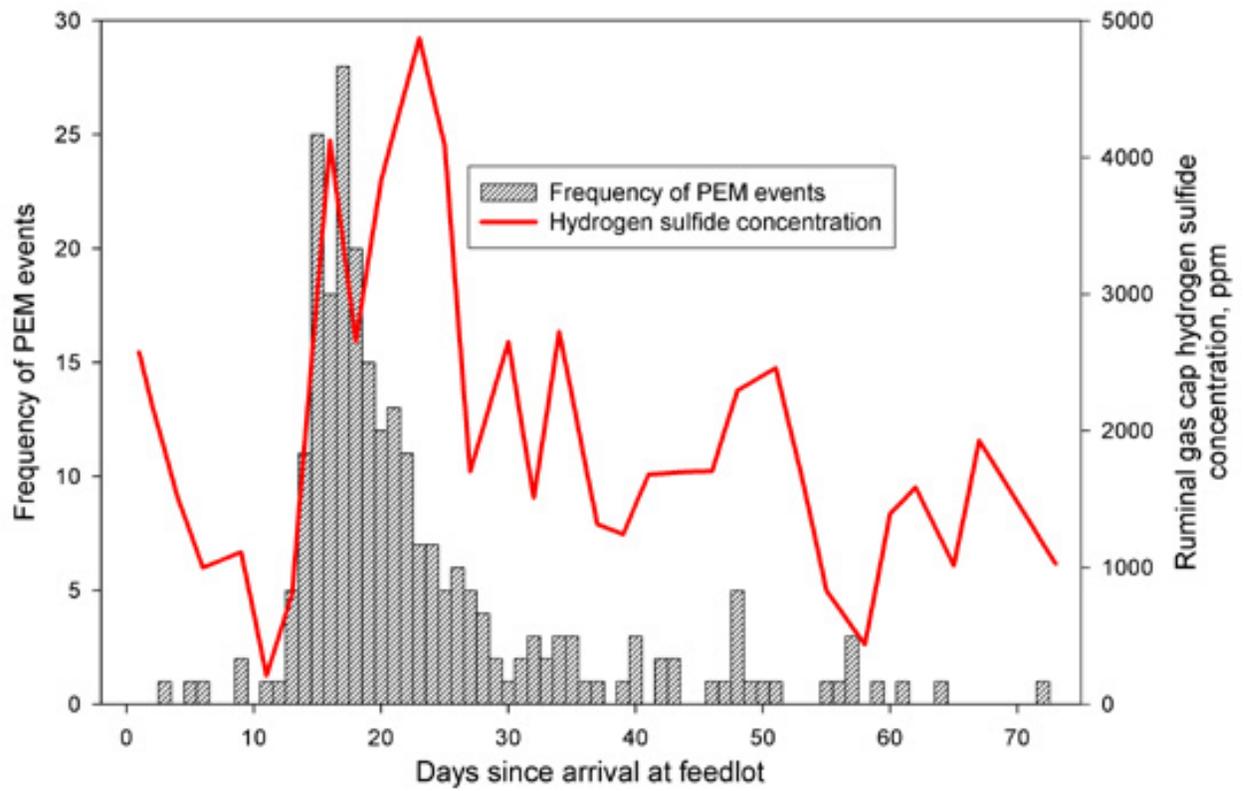
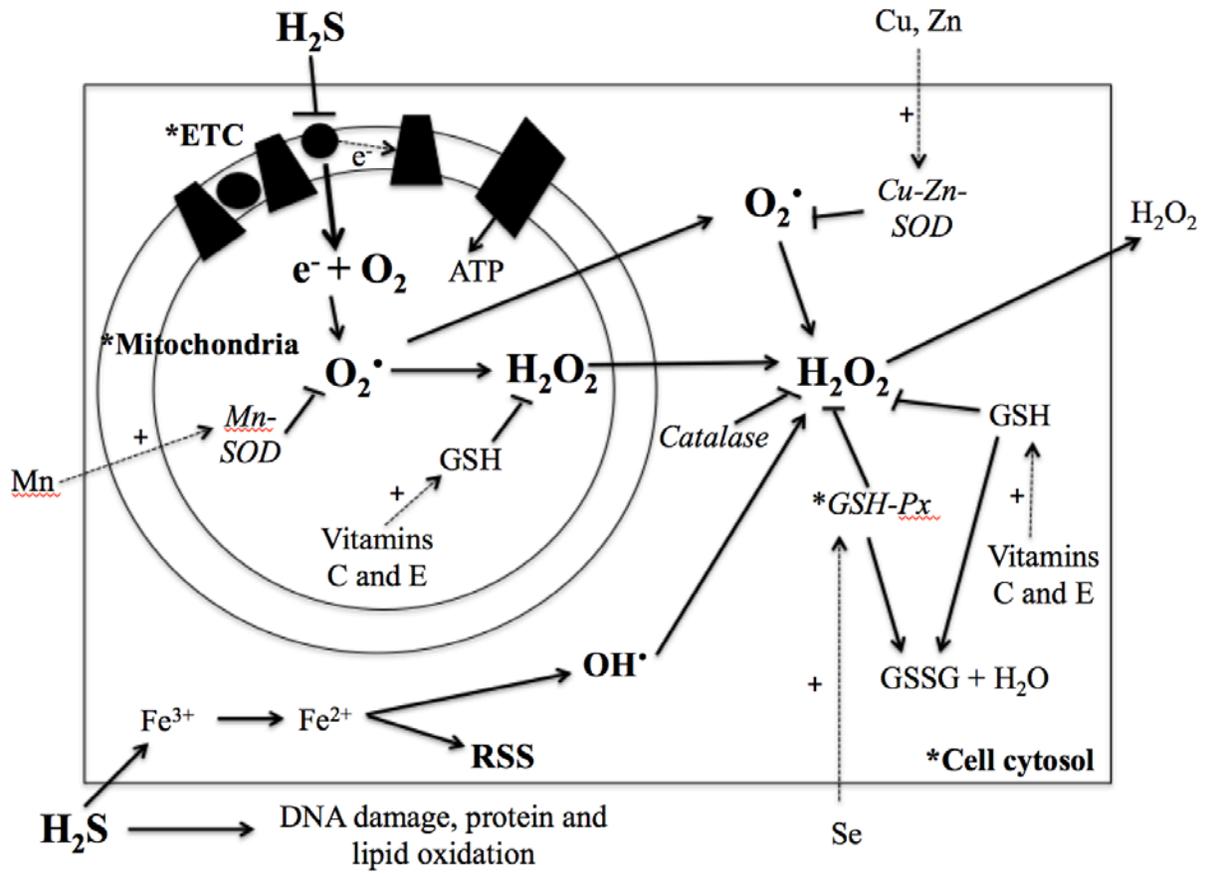


Figure 3.



1

2

Figure 4.

