THE ROLE OF DISTILLERS DRIED GRAINS WITH SOLUBLES IN SULFUR TOXICITY IN RUMINANTS

A Dissertation
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Bryan Wayne Neville

In Partial Fulfillment of the Requirements
for the Degree of
DOCTOR OF PHILOSOPHY

Major Department:
Animal Sciences

November 2010

Fargo, North Dakota

North Dakota State University
Graduate School

Title

The Role of Distillers Dried Grains with Solubles in Sulfur Toxicity in Ruminants

By

Bryan Wayne Neville

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

ABSTRACT

Neville, Bryan Wayne, Ph.D. Department of Animal Sciences, College of Agriculture, Food Systems, and Natural Resources, North Dakota State University, November 2010. The Role of Distillers Dried Grains with Solubles in Sulfur Toxicity in Ruminants. Major Professors: Dr. Christopher Schauer and Dr. Gregory Lardy.

One of the challenges with using ethanol co-products is the potential for increased dietary S concentration. Dietary S concentration has been implicated as a cause of polioencephalomalacia (**PEM**) in ruminants. The focus of this research was to evaluate PEM in ruminants fed distillers dried grains plus solubles (**DDGS**) based finishing rations. Two separate hypotheses were formed: 1) Providing increased dietary thiamin will decrease the incidence of PEM in lambs fed increased S diets without affecting animal performance; and 2) Feeding DDGS would increase concentrations of H_2S gas and incidence of PEM compared to diets based on dry-rolled corn. Two studies were conducted utilizing lambs to evaluate either 1) the influence of increasing supply of dietary thiamin (0, 50, 100, or 150 mg/d) on performance and incidence of PEM when fed diets containing 60% DDGS or 2) the influence of DDGS inclustion (0, 20, 40, or 60% DM basis) on sulfur balance. A third study was conducted using beef steers to examine the influence method of corn processing (high-moisture vs. dry-rolled corn) and concentration DDGS (20, 40, or 60% DM basis) on animal performance, H_2S concentrations and incidence of PEM. Hydrogen sulfide gas concentrations were measured via rumenocentesis as lambs and steers were adapted from a receiving diet to a finishing diet. No differences in lamb performance were noted ($P \geq 0.17$) when diets containing increasing concentrations of thiamin were fed. Sulfur excretion increased ($P \leq 0.01$) with increasing dietary DDGS. Lambs fed elevated concentrations of DDGS had a 3 fold increase in water intake and a 4.8 fold increase S excretion via urine compared to lambs fed no DDGS. Steer performance

decreased ($P \leq 0.02$) with increasing concentration of DDGS. Hydrogen sulfide gas concentrations did not differ ($P \geq 0.06$) until d 14 when lambs fed 60% DDGS had greater H_2S concentrations (0.23 ± 0.039 g/m^3; $P \leq 0.006$) than all other treatments. Lambs fed 60% DDGS continued to have greater ($P \leq 0.001$) H_2S gas concentrations throughout the adaptation phase compared to the other treatments. Lambs fed 150 mg of thiamin per day and steers fed 60% DDGS had the greatest concentrations of H_2S (1.07 g H_2S/m^3; $P \leq 0.009$; 1.38 g H_2S/m^3, $P \leq 0.01$), respectively). Ruminal H_2S concentrations in steers were affected by increasing DDGS concentration in the diet ($P < 0.001$), but not by corn processing method ($P = 0.94$). Ruminal pH was not affected by a day x treatment interaction ($P = 0.65$) or by treatment ($P = 0.32$), but decreased ($P < 0.001$) across the adaptation phase from 5.82 (d -7) to 5.33 (d 35) in lambs fed increasing concentrations of DDGS. The use of thiamin as a dietary additive to aid in the prevention of PEM in finishing lambs does not appear to be necessary under the conditions of this study. Corn processing did not influence animal performance or H_2S concentrations in our study. Steer performance decreased when DDGS level was > 40% (DM basis); however lambs fed 60% DDGS had similar performance compared to lambs fed lesser amounts of DDGS. The role dietary S from DDGS plays in incidence of PEM is questionable as no cases of PEM were observed with dietary S concentrations exceeding 2-3 times the maximum tolerable level. Additional research may be needed to clarify species specific observations and responses to dietary S levels.

ACKNOWLEDGMENTS

First and foremost I must thank my wife, Tammi, for all of her support over the past 5 years. Without her I would not have came to NDSU and built the friendships and professional relationships that I hope stay with me for the rest of my life. There has not been a single day that has gone by that I have not been grateful that you are by my side. All I can ask for is a lifetime filled with memories like the ones we have had in North Dakota.

Second, I would like to thank Dr. Greg Lardy whose guidance has been outstanding. I appreciate that Greg let me build my own reputation by allowing me to design my own research program and figure out my own way through graduate school. Additionally, I would like to thank Dr. Chris Schauer for his willingness to take on his first Ph.D. student in animal sciences. The experiences that Chris has passed on will be invaluable to me in the future.

I would also like to thank the undergraduates that helped me out with my research. Lesley Kirschten, I wish you all the best in your education at Iowa State University and I hope you have a very successful career as a veterinarian. Mary Berg, I also wish you luck in pursuing your Masters Degree at the University of Wisconsin. Paul Brown and Nick Keena thank you for being there when I needed help. I could always count on you to get the work done no matter the situation. And finally, Kelcey Holm you always know how to cheer up a room, thank you for all the hard work. Thank you to all of you for helping make my graduate program a success.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS

ADF.....Acid detergent fiber

ADG.....Average daily gain

AOAC.....Association of Official Analytical Chemists

APS.....Adenosine 5'-phosphosulfate

ATP.....Adenosine triphosphate

BCTRC.....Boneless closely-trimmed retail cuts

BW.....Body weight

C.....Celsius

Ca.....Calcium

cm.....Centimeter

CP.....Crude protein

Cu.....Copper

CSB.....Concentrated separator byproduct

d.....Day

DDGS.....Distillers dried grains with solubles

diam.....Diameter

DM.....Dry matter

DMI.....Dry matter intake

DRC.....Dry-rolled corn

g.....Gram(s)

g/m^3.....Grams per cubic meter

G:F.....Gain-to-feed ratio

h.....Hour

hd.....Head

HCW.....Hot carcass weight

HMC.....High-moisture corn

HPLC.....High performance liquid chromatography

H_2S.....Hydrogen sulfide

IU.....International unit

kg.....Kilogram(s)

KPH.....Kidney, pelvic, and heart fat

L.....Liter(s)

mg.....Milligram(s)

mL.....Milliliter(s)

mm.....Millimeter(s)

mo.....Month

n.....Number

N.....Nitrogen

NDF.....Neutral detergent fiber

NDSU.....North Dakota State University

NE.....Net energy

NRC.....National Research Council

P.....Phosphorus

PAPS.....3' Phospho-adenosine 5'-phosphosulfate

PEM.....Polioencephalomalacia

ppm.....Parts per million

S.....Sulfur

SAS.....Statistical Analysis Software

VFA.....Volatile fatty acid

WDGS.....Wet distillers grains with solubles

wt.....Weight

wk.....Week

w/v.....Weight per volume

Zn.....Zinc

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Introduction

Distillers dried grains with solubles (**DDGS**) are an important feed source in both lamb and beef finishing operations. A survey of feedlot consultants (Vasconcelos and Galyean, 2007) reported that 82.76% of clients used distillers grains in finishing diets with a range of DDGS inclusion from 5 to 50% (DM basis). Distillers grains produced from the fuel-ethanol industry have and will continue to be the topic of research as long as public perception and policy dictate a need for grain ethanol. Wisner (2010) estimates that nearly 40 million tons of distillers grains will be produced annually in 2010 and 2011 as a result of corn-ethanol production. Corn is used as a major source of dietary energy in cattle and sheep finishing diets. Due to competition for corn between ethanol production and the livestock industry improving the use of DDGS in finishing diets is a natural progression towards decreasing market competition for feed and/or energy resources.

Use of high sulfur feeds, such as DDGS from the ethanol industry, has led to an increased interest in sulfur toxicity. Fermentation of corn to produce ethanol naturally concentrates the S found in corn. During the production of ethanol various sulfur containing acids are added in to improve efficiency of fermentation and simultaneously increasing ethanol yield. As a consequence, S concentration of DDGS can range from 0.6 to 1.0% S (DM basis; Klopfenstein et al., 2008). These S concentrations increase the risk of polioencephalomalacia (**PEM**) in finishing lambs and cattle. Polioencephalomalacia is a condition characterized by cerebral necrosis and can be caused by elevated sulfur intakes (McDowell, 2003). Symptoms of PEM include: ataxia, blindness, and seizures which can

be followed by death (NRC, 2005). Other causes of PEM include thiamin deficiency, lead poisoning, and water depravation/salt toxicity.

Hydrogen sulfide and sulfur dioxide are produced within the rumen during fermentation by the rumen microoganisms. These gasses enter the lungs during eructation and lead to central nervous system disruption (Dougherty et al., 1965). Further, research has demonstrated that sulfur dioxide can cleave thiamin (NRC, 2005) resulting in thiamin deficiency which potentially leads to cerebrocortical necrosis in ruminants (Edwin and Jackman, 1982). Lesions originating from S toxicity are similar, but not identical, to cerebrocortical necrosis lesions found with thiamin deficiency (Jeffrey et al., 1994) which makes distinguishing between lesions formed as a result of S toxicity and thiamin deficiency is difficult.

The following literature review will outline the following topics: 1) how S in distillers grains is concentrated during the production to ethanol, 2) research conducted feeding elevated concentrations of S in lamb and beef cattle finishing diets, and 3) pertinent research on causes and treatment of S toxicosis in ruminant animals.

Literature Review

Production of Ethanol and Ethanol By-products

The production of ethanol from corn, emphasizing areas in which sulfur is either concentrated or added, is outlined in Figure 1.1. Ethanol production from corn begins by grinding corn in a hammer mill resulting in a fine ground corn or 'mill'. The mill is then combined with water and alpha-amylase and heated to liquefy starch. Temperatures at this stage are 120-150 degrees Celsius; lower temperature holding periods (95 degrees Celsius) may be used to reduce bacteria levels in the resulting mash (Berger and Singh, 2010).

Saccharification involves cooling the mash and adding glucose-amylase to convert the liquefied starch to dextrose. Yeast is then added to the mash to produce ethanol and carbon dioxide.

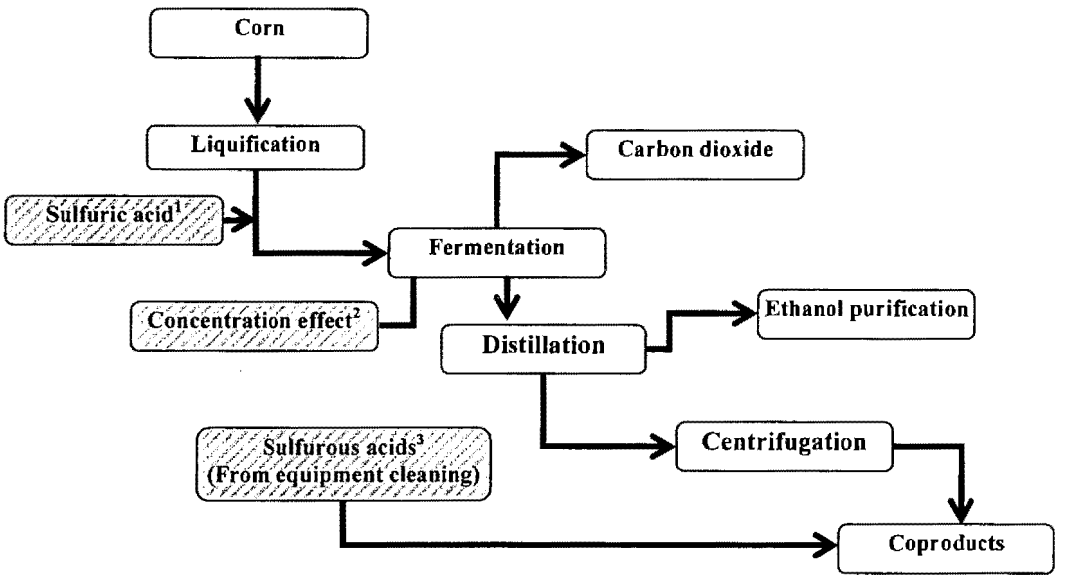

Figure 1.1. Flow diagram outlining production of ethanol from corn. Cross-hatch boxes indicate areas where sulfur is added to distillers dried grains with solubles via sulfurous acids or increased by removal of starch from corn. Adapted from: Berger and Singh (2010). [1]Sulfuric acid is added to decrease pH which optimizes fermentation of starch to ethanol. [2]Sulfur along with the other nutrients in corn are concentrated proportionally due to fermentation of starch to ethanol and its subsequent removal. [3]Sulfurous acids are also used to clean equipment decreasing opportunity for secondary fermentation and contamination.

After fermentation the mixture is referred to as 'beer'. The beer is then separated into two components, one containing water and ethanol the other containing the solids (i.e. yeast and non-fermentable fraction of the corn) and water. The distillation and dehydration process then removes the mash and water resulting in pure ethanol. Separation of solids begins the process of forming final products. There are three products formed in the production of ethanol: ethanol, carbon dioxide, and distillers grains (Lardy, 2007). The

mixture of yeast, non-fermentable corn, and water is called whole stillage. Whole stillage is centrifuged to produce thin stillage and wet grains (Berger and Singh, 2010). Thin stillage, a mixture of water and soluble solids, is then concentrated by passing the material through an evaporator to produce a co-product called condensed distillers solubles or 'distillers syrup'. Distillers syrup can then be added back to the wet grains to produce wet distillers grains with solubles (WDGS). Wet distillers grains with solubles can be dried resulting in DDGS.

Sulfur in DDGS comes from two sources, endogenous S contained in corn as well as sulfurous acids which are added to regulate fermentation and clean equipment in the distillation phase (Vannes et al., 2009). Sulfur, along with the other nutrients in corn, are concentrated proportionally due to fermentation of starch to ethanol. This is termed the 'concentration effect' and increases concentration of nutrients approximately 3-fold. For example, corn contains 9% CP while DDGS contains approximately 30% CP. Sulfuric acid is also added to the beer to regulate pH during fermentation and is also used to clean equipment and prevent unwanted fermentation. The pH of mash during fermentation must be lowered to 6.0 (Bothast and Schlicher, 2005) to activate enzymes and improve fermentation. Sulfuric acid is used to regulate pH during fermentation. Sulfuric acid is preferred over other acids, such as phosphoric acid, due to cost, availability, and effectiveness. In addition, using phosphoric acid increases the P concentration in DDGS even further as P is already high in DDGS (Vannes et al., 2009).

Theories Regarding S Toxicity

Thiamin deficiency. Historically, PEM has been thought to arise from a deficiency of thiamin. The structure of thiamin is depicted in Figure 1.2. Sulfur may negatively

impact thiamin by cleaving thiamin at the methylene bridge between the pyrimidine and thiazole rings mimicking the action of thiaminase (McDowell, 2000). In addition to thiaminase activity, thiamin-destroying activity in rumen can be increased by sulfate (Olkowski et al., 1993). Interestingly, while sulfate increases thiamin destruction in the rumen, sulfate does not impact ruminal synthesis of thiamin (Olkowski et al., 1993).

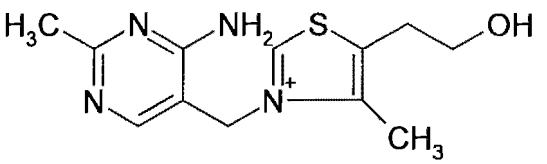

Figure 1.2. Structure of thiamin. Adapted from L. R. McDowell (2000) Vitamins in Animal and Human Nutrition.

There are two types of thiaminases, Type I and II (Brent and Bartley, 1984). Type II thiaminases cleave thiamin at the methylene bridge between the thiazole and pyrimidine rings; while type I thiaminases substitutes the thiazole ring completely with a nitrogen-containing ring (McDowell, 2000). In addition to destroying thiamin, thiaminase I activity produces thiamin analogs which are composed of the pyrimidine ring and a portion of a co-substrate from the original thiamin; these analogs inhibit energy metabolism in the central nervous system (Frye et al., 1991).

Thiamin deficiency can be treated or prevented with thiamin supplementation if caught in the early stages of deficiency. Injectable thiamin (1 g/d) given until the animals regain appetite followed by (500 mg/d) thiamin in the diet for 7-14 will alleviate the effects of PEM (Mathison, 1986). Mathison (1986) further recommended feeding 4-6 mg thiamin per kg dry feed to prevent subclinical deficiency when feeding high concentrate diets. Loneragan et al. (2005) hypothesized that the therapeutic effects of thiamin in PEM-

affected animals may be attributed to either an increased requirement for thiamin or a beneficial effect of thiamin on impaired brains. Source of supplemental thiamin is also important; the mononitrate form is preferred as it is more stable than the hydrochloride form (Bauernfeind, 1969). Given that excess thiamin is cleared by the kidneys (McDowell, 2000) and levels nearly 1000 times requirement are thought to be safe (NRC, 1987), thiamin toxicity is extremely rare and minimizes risks of over-supplementation of thiamin. Thiamin toxicity is prevented by increased urinary excretion of thiamin until a point of saturation, at which time additional thiamin is excreted in the feces (Bräunlich and Zintzen, 1976).

A review of recent literature demonstrates that supplementation with thiamin does not guarantee prevention of PEM when feeding high S diets to ruminants. Olkowski et al. (1992) reported that outward clinical signs of PEM in lambs fed high sulfur diets (0.63% S) were prevented by supplementing 243 mg thiamin/kg dietary DM. However, microscopic lesions in brain tissue were not totally prevented (Olkowski et al., 1992). Schauer et al. (2008) reported lambs which were fed up to 60% DDGS (0.55% S; DM basis) and which received 142 $mg \cdot hd^{-1} \cdot d^{-1}$ of supplemental thiamin had no incidence of PEM. Huls et al. (2008) reported that steers fed 50% modified distillers grains plus solubles (DM basis) and supplemented with 150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin performed similarly to steers fed a diet based on high-moisture corn and dry-rolled corn-based diet containing no distillers grains. While Huls et al. (2008) reported no incidence of PEM they did not report criteria for determination of PEM or S concentration of the diet. This raises the question of to what degree this study measured incidence of PEM. Contrary to these results, Buckner et al. (2007) discontinued feeding a treatment diet which contained 50% DDGS (0.60% S; DM

basis) when multiple steers either died or exhibited visual signs of PEM while receiving 150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin. These inconsistencies demonstrate the need for additional research to determine the appropriate amount of thiamin needed to prevent PEM when feeding elevated amounts of S. Further, this research assumes that thiamin supplementation has an overriding effect on S induced PEM. While this has not been demonstrated in the literature, it is widely accepted dogma.

Adaptation of ruminant animals from medium concentrate to high concentrate diets that contain high concentrations of S is of great importance as the combination of decreasing pH and increasing sulfur supply may lead to PEM. The link between dietary sulfur and ruminal pH change have been reviewed by Gould (1998), who concluded that in diets with S concentrations exceeding 0.3%, the combination of dietary S concentration, ruminal sulfide production, and increased thiaminase production may increase incidence of PEM. Alves de Oliveria et al. (1996) reported decreased ruminal pH does not decrease microbial production of thiamin; however, the decrease in rumen pH increases populations of thiaminase producing bacteria (Morgan and Lawson, 1974; Boyd and Walton, 1977; Thomas et al., 1987).

Hydrogen sulfide toxicity. Hydrogen sulfide is a toxic compound that rivals cyanide in terms of toxicity (NRC, 1980). Kandylis (1984) reported that H_2S present in the rumen may cause neurological or respiratory distress. Sulfur reducing bacteria in the rumen have the ability to reduce inorganic sulfur to sulfide thus generating H_2S. Production of ruminal sulfide is facilitated by sulfur reducing bacteria such as *Desulfovibrio spc.* Sulfur entering the rumen as sulfate enters these bacteria and is converted to either adenosine-5'-phosphosulfate (**APS**) or 3'-phosphoadenosine-5'-phosphosulfate (**PAPS**). These

molecules represent the starting points for the dissimilatory and assimilatory sulfur reduction pathways (Figure 1.3).

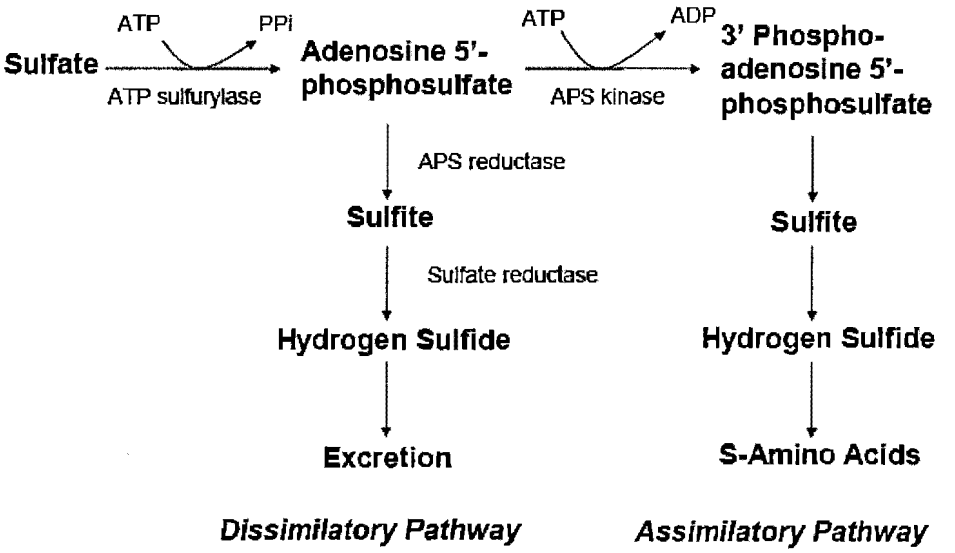

Figure 1.3. Dissimilatory and assimilatory pathways of rumen sulfate reducing bacteria. Adapted from Kung (2008). Assimilatory sulfate reducing bacteria do not produce detectable amounts of H_2S and reduce only enough sulfate to meet S requirements. Dissimilatory sulfate reducing bacteria utilizes sulfate a terminal electron receptor. The presence of APS-reductase dictates which reduction pathway is utilized.

Adenosine-5'-phosphosulfate is then reduced to form sulfite and subsequently sulfide. Sulfide can then serve as a hydrogen sink and accept the 2 hydrogen molecules required to form hydrogen sulfide. The assimilatory pathway is less energy efficient as it requires an additional ATP to convert APS to PAPS. This conversion is required as dissimilatory sulfate reducing bacteria can produce the enzyme APS-reductase whereas assimilatory sulfate reducing bacteria cannot produce this enzyme (Peck, 1961). Further, assimilatory sulfate reducing bacteria only reduce enough sulfate to meet nutritional requirements (Peck, 1961). There are multiple forms of sulfur containing compounds leaving the rumen, including sulfur containing amino acids, sulfide, and hydrogen sulfide.

Sulfur containing amino acids such as methionine, cysteine, and taurine flow to the small intestine where they are absorbed. Sulfide can pass through the rumen wall into the blood, while hydrogen sulfide cannot pass through the rumen wall (NRC, 2005). This means that hydrogen sulfide accumulated in the rumen gas cap and is expelled with the other fermentation gases during eructation.

Hydrogen sulfide is believed to enter the body following eructation by being inhaled into the lungs where it is passively absorbed into the blood. Once in the blood, H_2S can act as an inhibitor of oxygen transport by competitively inhibiting oxygen binding of hemoglobin, or interfere with energy metabolism at a cellular level by inhibiting the processes of cytochrome oxidase in the electron transport chain.

A definitive exposure level to H_2S has not been established for livestock. However, humans will experience respiratory paralaysis if exposed to 500-1000 ppm H_2S (Reiffenstein et al., 1992). Loneragan et al. (2005) reported that feedlot steers receiving water containing 2,360 mg sulfate/L had peak ruminal H_2S gas concentrations between 5500 and 6000 ppm. Likewise, Niles et al. (2002) reported steers consuming a corn gluten feed-based ration with 0.7% S had ruminal gas cap H_2S concentrations of 18,642 ppm on d 28 of their study. Both studies reported cattle suffering from PEM based on histological analysis of brain tissue.

Feeding highly fermentable carbohydrates, such as high-moisture corn or steam-flaked corn, can decrease ruminal pH. However, steam-flaked corn did not increase in vitro H_2S production compared to dry-rolled corn (Leibovich et al., 2009). Leibovich et al. (2009) stated that an increase in available starch does not increase H_2S gas production. However Leibovich's work focused on low dietary S content (0.2 – 0.25% S) and the

impact greater concentrations of S (0.6 – 0.9%) deserves exploration. Previous work (May et al., 2009) indicated changes in ruminal ammonia, pH, and VFA concentrations do occur within a 24 h period when various concentrations of DDGS and corn processing methods are utilized in ruminant diets. As a result, changes in hydrogen sulfide concentration over the course of a single 24 h period may be affected by corn processing methods.

The adaptation phase, that time when ruminant animals are transitioned from high roughage diets to high concentrate diets, is of particular interest in terms of H_2S production. Gould et al. (1997) indicated that H_2S concentrations decrease after adaptation to high concentrate rations. Differences in acute versus chronic exposure to S may exist. Depending on type of exposure rumen microbes may adapt to increased amounts of S and increase sulfate reduction pathways. Other work (Niles et al., 2002) feeding various concentrations of co-products demonstrated that increasing DDGS concentration in the diet increased H_2S concentration in the rumen gas cap.

Lacking in the literature are direct comparisons between different species such as sheep and cattle; these comparisons are difficult to establish as management practices such as inclusion of various feed additives may (Kung et al., 2000) or may not (Quinn et al., 2009) influence H_2S production by rumen bacteria. Compounds which influence rumen metabolism and H_2S production include chlortetracycline, oxytetracycline, and monesin (Kung et al., 2000). Other explanations for differences in H_2S concentration were offered by Gould (1998) and include sulfide contained in ruminal fluid, ruminal fluid pH, frequency of eructation, and absorption of sulfide through the rumen mucosa. Clearly, defining S source (water vs. feed) as well as form (organic vs. inorganic) as determining factors influencing S toxicity is needed in the scientific literature. Accounting for

digestibility or availability of various S sources will facilitate a more appropriately definition of both maximum tolerable and toxic levels of S in future recommendations.

Effects of S on Animal Performance

Dry matter intake. Dry matter intake directly influences animal performance. A meta-analysis of wet distillers grains with solubles (**WDGS**) feeding studies demonstrated that DMI decreased as WDGS inclusion increased up to 50% dietary DM when replacing DRC (Klopfenstein et al. 2008). The same meta-analysis reported an increase in DMI when DDGS was fed at levels up to 40%. However, data regarding DMI when feeding DDGS are variable. Decreases in ruminal and intestinal motility (Bird, 1972; Kandylis, 1984) due to S content could explain the decreased DMI observed with increasing DDGS inclusion. Loneragan et al. (2001) hypothesized that either decreased gut motility or hepatic injury may reduce animal performance. However, hepatic injury may be influenced by management and use of feed additives, such as Tylosin (Nagaraja and Chengappa, 1998; Vasconcelos and Galyean, 2008).

Corrigan et al. (2009) reported that independent effects of WDGS inclusion level and corn processing method on DMI. However, as indicated previously, there is likely variation due to type of distillers grains with solubles (wet vs. dry) as well as the corn processing methods, possibly explaining differences present in the literature. Whether the decreases in intake and performance are a function of hydrogen sulfide decreasing gut motility or causing sub-clinical PEM is unknown. However, Loneragan et al. (2001) concluded that while hydrogen sulfide levels may not be great enough to cause toxicity, high H_2S may decrease productivity. Loneragan et al. (2001) did not report how much H_2S

is required to affect animal performance or cause PEM. Further research is needed to confirm these conclusions.

Carcass characteristics. Carcass characteristics may also be impacted by inclusion of DDGS. Corrigan et al. (2009) reported improved carcass characteristics and feedlot performance with increasing wet distillers grains with solubles inclusion compared to a corn based diet. Although Corrigan's data demonstrated improved performance when increasing dietary distillers grains up to 40% the ration, the negative relationship associated with feeding over 40% distillers grains has not been explored in great detail.

If S content is considered independent of source, e.g. DDGS vs. water sulfates, the inconsistencies within the literature become more apparent. Zinn et al. (1997) demonstrated that dietary S concentrations of 0.25% S (DM basis) decreased performance and carcass merit in cattle when the S source was ammonium sulfate. Previous work (Smith et al., 1964) reported that lambs receiving 0.43% S, in the form of elemental S, had greater carcass quality compared to lambs fed either 0.13 or 1.3% S. Schauer et al. (2008) reported lambs having greater flank streaking when feeding increasing concentrations of DDGS. Schauer attributed the increases in carcass quality to increases in energy density of the diet.

Incidence of PEM. A review of literature reporting the amount of S fed to ruminants in corn byproduct-based rations simply confirms the inconsistencies in the amount of S required to cause neurological problems such as PEM. The incidence rate of PEM was reported as 0.1% when feeding less than 20% co-products ($\leq$ 0.46% S DM basis; Vannes et al., 2009). Vannes et al. (2009) also reported that feeding diets containing greater than 0.56% S raised PEM incidence to 6.06%. Previous research (Loza et al., 2010)

reported that 12 steers were diagnosed with PEM when fed a diet containing 75% blended wet corn gluten feed and WDGS (0.45% S; DM basis); however this diet contained no roughage. Krasicka et al. (1999) reported that all lambs fed a low fiber-high starch diet containing 0.72% S died from PEM after 12 weeks. Schauer et al. (2008) fed lambs a finishing diet which contained 0, 20, 40, or 60% DDGS and reported no incidences of PEM; these diets contained up to 0.55% S (DM basis). Niles et al. (2002) reported that 10 of 14 calves fed a corn gluten feed-based diet exhibited PEM; those calves affected were fed diets that contained either 0.55 or 0.70% S (DM basis). Both Schauer and Niles reported water sulfate values; the water consumed by the lambs (Schauer et al., 2008) contained 141 mg sulfate/L, while the water consumed by the steers (Niles et al., 2002) contained 56 mg sulfate/L. Unfortunately, Niles et al. (2002) did not report how much, if any, supplemental thiamin was provided to the steers in their study; however, Schauer et al. (2008) reported lambs in their study received 142 $mg \cdot hd^{-1} \cdot d^{-1}$ of supplemental thiamin. Huls et al. (2008) reported that steers fed 50% modified distillers grains plus solubles (DM basis) and supplemented with 150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin performed similarly to steers fed a high-moisture corn and dry-rolled corn-based diet. Contrary to Huls' results, Buckner et al. (2007) discontinued feeding a treatment diet which contained 50% DDGS (0.60% S; DM basis) when multiple steers exhibited PEM while receiving 150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin.

Sulfur from water sources has also been implicated as a cause of PEM in ruminants. Ward and Paterson (2004) evaluated thiamin supplementation as a method of preventing PEM in steers consuming high sulfate (4000 mg/L) water. Two steers (approx. 10% of steers) on high sulfate water and one steer (approx. 5% of steers) from high sulfate water supplemented with 1000 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin died; however, only one of the cases from the

unsupplemented group was confirmed to have died from PEM. Cammack et al. (2010) also reported 12 cases (12.5% incidence of PEM) of sulfur induced PEM in steers consuming high sulfate water. Although no incidences of PEM occurred, Loneragan et al. (2001) reported consuming water with increasing sulfate concentrations negatively impacted steer performance and carcass characteristics. However, no differences in performance were observed in lambs fed DDGS diets containing increasing amount of S from DDGS (Schauer et al., 2008).

Conclusions

There is a lack of data in the scientific literature regarding the various forms of S contained within DDGS. Quantifying proportions of the various forms of S will undoubtedly add to the current literature and assist in determination of mechanisms of S-toxicity in the ruminant animal. Determining how digestibility or availability of S in its various forms influences S reduction and creation of H_2S gas within the rumen will further aid in the understanding S-toxicity mechanisms. We therefore recommend that the role DDGS play in sulfur toxicity in the ruminant animal be evaluated in more detail. Further, this review illustrates the need for additional research to further determine the interactive affects of S, thiamin supplementation, and dietary grain concentration in finishing rations, and the effect they collectively have on the incidence of PEM. In addition, research is needed to evaluate these practices in cattle and to determine the effect of the form of S in the feed and water as well as its fate in the animal (absorption, retention, and excretion). It is possible that cattle and sheep have different tolerances for excess S or different tolerances for ruminal H_2S. Consequently research is needed to confirm whether or not sheep are a good model for cattle with regard to S induced PEM. Finally, distinction

between maximum tolerable level and toxicity is needed within the NRC and scientific literature.

Literature Cited

Alves de Oliveria, L., D. Jean-Blain, A. Durix, S. Komisarczuk-Bony, and C. Durier. 1996. Use of a semi-continuous culture system (RUSITEC) to study the effect of pH on microbial metabolism of thiamin (Vitamin B1). Arch. Anim. Nutr. 49:193-202.

Bauernfeind, J. C. 1969. World Rev. Anim. Prod. 5(21):20.

Berger, L. and V. Singh. 2010. Changes and evolution of corn coproducts for beef cattle. J. Anim. Sci. 88(Suppl.):E143-E150.

Bird, P. R. 1972. Sulphur metabolism and excretion studies in ruminants X. Sulphide toxicity in sheep. Aust. J. Biol. Sci. 25:1073-1085.

Bothast, R. J., and M. A. Schlicher. 2005. Biotechnological processes for conversion of corn into ethanol. Appl. Microbiol. Biotechnol. 67:19-25.

Boyd, J. W., and J. R. Walton. 1977. Cerebrocortical necrosis in ruminants: An attempt to identify the source of thiaminase in affected animals. J. Comp. Pathol. 87:581-589.

Bräunlich, K., and H. Zintzen. 1976. Vitamin B1 No. 1593. Hoffmann-La Roche, Basel, Switzerland.

Brent, B. E., and E. E. Bartley. 1984. Thiamin and niacin in the rumen. J. Anim. Sci. 59:813-822.

Buckner, C. D., T. L. Mader, G. E. Erickson, S. L. Colgen, K. K. Karges, and M. L. Gibson. 2007. Optimum concentrations of dry distillers grains with solubles for finishing beef steers. 2007 Nebraska Beef Report. MP90:36-38.

Cammack, K. M., C. L. Wright, K. H. 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 forages-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. Vannes, and K. J. Hanford. 2009. Effect of corn processing method and corn wet distillers grains plus solubles inclusion level in finishing steers. J. Anim. Sci. 87:3351-3362.

Dougherty, R. W., C. H. Mullenax, and M. J. Allison. 1965. Physiological phenomena associated with eructation in ruminants. In: Physiology of digestion in the ruminant. Butterworths. Washington, D.C.

Edwin, E. E. and R. Jackman. 1982. Ruminant thiamine requirement in perspective. Vet. Res. Commun. 5:237-250.

Frye, T. M., S. N. Williams, and W. Graham. 1991. Vitamin deficiencies in cattle. Vet. Clin. North Am. Food Anim. Pract. 7:217-275.

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

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

Huls, T. J., M. K. Luebbe, G. E. Erickson, and T. J. Klopfenstein. 2008. Effect of inclusion level of modified distillers grains plus solubles in finishing steers. 2008 Nebraska Beef Report. MP91:41-42.

Jeffrey, M., J. P. Duff, R. J. Higgins, C. R. Simpson, R. Jackman, T. O. Jones, S. C. Mechie, and C. T. Livesey. 1994. Polioencephalomalacia associated with the ingestion of ammonium sulphate by sheep and cattle. Vet. Rec. 34:343-348.

Kandylis, K. 1984. Toxicology of sulfur 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 by-products in the beef cattle feeding industry. J. Anim. Sci. 86:1223-1231.

Krasicka, B, M. A., Gralak, B. Sieranska, and G. Kulasek. 1999. The influence of dietary sulphur loading on metabolism and health in young sheep fed low fibre and high starch diet. Reprod. Nutr. Dev. 39:625-636.

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.

Kung, L. 2008. Metabolism of sulfur in the rumen. *Presentation* Midwest Sec. Am. Soc. Anim. Sci. Des Moines, IA.

Lardy, G. P. 2007. Feeding coproducts of the ethanol industry to beef cattle. North Dakota State University. AS-1242.

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

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.

Loneragan, G, D. Gould, J. Wagner, F. Garry, and M. Thoren. 2005. The magnitude and patterns of ruminal hydrogen sulfide production, blood thiamin concentration, and mean pulmonary arterial pressure in feedlot steers consuming water of different sulfate concentrations. Bov. Pract. 39:16-22.

Loza, P. L., C. D. Buckner, K. J. Vander Pol, G. E. Erickson, T. J. Klophenstein, and R. A. Stock. 2010. Effect of feeding combinations of wet distillers grains and wet corn gluten feed to feedlot cattle. J. Anim. Sci. 88:1061-1072.

Mathison, G. W. 1986. Proc., 21st Northwest Anim. Nutr. Conf. Vancouver, Canada.

May, M. L., 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 twenty-five percent dried distillers grains on ruminal fermentation and apparent total tract digestion. J. Anim. Sci. 87:3630-3638.

McDowell, L. R. 2000. Vitamins in animal and human nutrition. 2nd Edition. Iowa State University Press. Ames, IA.

McDowell, L. R. 2003. Minerals in animal and human nutrition 2nd Ed. Elsevier Science. Amsterdam, The Netherlands.

Morgan, K. T., and G. H. Lawson. 1974. Thiaminase type 1-producing bacilli and ovine polioencephalomalacia. Vet. Rec. 95:361-363.

Nagaraja T. G., and M. M. Chengappa. 1998. Liver abscesses in feedlot cattle: A review. J. Anim. Sci. 76:287-298.

National Research Council. 1980. Mineral tolerance of domestic animals. The National Academies Press. Washington, D.C.

National Research Council. 1987. Vitamin tolerances or animals. National Academy of Science-National Research Council, Washington, D.C.

National Research Council. 2005. Mineral tolerances of animals, Second revised edition. The National Academies Press. Washington, D.C.

Niles, G. A., S. Morgan, W. C. Edwards, and D. Lalman. 2002. Effects of dietary sulfur concentrations on the incidence and pathology of polioencephalomalacia in weaned beef calves. Vet. Human Toxicol. 44(2):70-72.

Olkowski, A. A., S. R. Gooneratne, C. G. Rousseaux, and D. A. Christensen. 1992. Role of thiamine status in sulphur induced polioencephalomalacia in sheep. Res. Vet. Sci. 52:78.

Olkowski, A. A., B. Laarvold, J. F. Patience, S. I. Francis, and D. A. Christensen. 1993. The effect of sulfate on thiamine-destroying activity in rumen content cultures in-vitro. Int. J. Vit. Nutr. Res. 63:38-44.

Peck, H. D., JR. 1961. Enzymatic basis for assimilatory and dissimilatory sulfate reduction. J. Bacteriol. 82(6):933-939.

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.

Reiffenstein, R. J., W. C. Hulbert, and S. H. Roth. 1992. Toxicology of hydrogen sulfide. Annu. Rev. Pharmacol. Toxicol. 32:109-134.

Schauer, C. S., M. M. Stamm, T. D. Maddock, and P. B. Berg. 2008. Feeding 60% of lamb finishing rations as dried distillers grains with solubles results in acceptable performance and carcass quality. Sheep and Goat Res. J. 23:15-19.

Smith, G. C., M. W. Galgen, and M. Weller. 1964. Effect of elemental sulfur on lamb performance and carcass quality. J. Anim. Sci. 23:892. (Abst.).

Thomas, K. W., D. L. Turner, and E. M. Spicer. 1987. Thiamine, thiaminase and transketolase levels in goats with and without polioencephalomalacia. Aust. Vet. J. 64:126-127.

Vannes, S., G. E. Erickson, T. J. Klopfenstein, and K. K. Karges. 2009. Sulfur in distillers grains. University of Nebraska Lincoln. Nebraska Beef Cattle Reports. p.79-80.

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.

Vasconcelos, J. T., and M. L. Galyean. 2008. ASAS Centennial Paper: Contributions in the Journal of Animal Science to understanding cattle metabolic and digestive disorders. J. Anim. Sci. 86:1711-1721.

Ward, E. H., and H. H. Patterson. 2004. Effects of thiamin supplementation on performance and health of growing steers consuming high sulfate water. Proc. West. Sec. Am. Soc. Anim. Sci. 55:375-378.

Wisner, R. 2010. Estimated distillers dried grains production and use. Ag Marketing Resource Center. Iowa State University. Ames, IA.

Zinn, R. A., E. Alvarez, M. Mendez, M. Montaño, 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.

CHAPTER II. EFFECT OF THIAMINE CONCENTRATION ON ANIMAL HEALTH, FEEDLOT PERFORMANCE, CARCASS CHARACTERISTICS, AND RUMINAL HYDROGEN SULFIDE CONCENTRATIONS IN LAMBS FED DIETS BASED ON 60% DISTILLERS DRIED GRAINS PLUS SOLUBLES

Abstract

Limited data is available regarding the influence of thiamin supplementation on incidence of polioencephalomalacia (**PEM**) in lambs fed diets containing high dietary S concentrations (> 0.7%). Therefore, our objective was to evaluate the influence of thiamin supplementation on feedlot performance, carcass quality, ruminal hydrogen sulfide gas concentrations, and incidence of PEM in lambs fed a finishing diet containing 60% distillers dried grains with solubles (**DDGS**; DM basis). Two studies were conducted using completely randomized designs to evaluate the influence of concentration of thiamin supplementation. Study 1 utilized 240 lambs fed in 16 pens while Study 2 utilized 55 individually fed lambs. Lamb finishing diets contained 60% DDGS which resulted in dietary S concentration of 0.73% (DM basis). Treatments diets were based on amount of supplemental thiamin provided, 1) no supplemental thiamin (**CON**), 2) 50 $mg \cdot hd^{-1} \cdot d^{-1}$ (**LOW**), 3) 100 $mg \cdot hd^{-1} \cdot d^{-1}$ (**MED**), or 4) 150 $mg \cdot hd^{-1} \cdot d^{-1}$ (**HIGH**). Additionally in Study 2, a fifth treatment was included which contained 0.87% S (DM basis; increased S provided by addition of dilute sulfuric acid) and provided 150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin (**HIGH+S**). In study 1, ADG decreased quadratically ($P = 0.04$) with lambs fed CON, LOW, and MED gaining faster than lambs fed HIGH. In Study 1, DMI responded quadratically ($P < 0.01$), while G:F tended to differ linearly ($P = 0.08$) to concentration of thiamin supplementation with MED lambs having greater DMI and decreased G:F. No differences ($P \geq 0.17$) in lamb performance were observed in Study 2. In both studies, most carcass characteristics were unaffected with the exception of a tendency for poorer carcass conformation (Study 1;

$P = 0.09$) and greater flank streaking (Study 2; $P = 0.03$). No differences in ruminal H_2S concentration ($P > 0.05$) among treatments were apparent until d 10, at which point lambs fed LOW had lower H_2S concentrations than all other treatments. Lambs fed HIGH had the greatest concentrations of H_2S on d 31 (1.07 g H_2S/m^3; $P < 0.009$). Ruminal pH did not differ ($P = 0.13$) and averaged 5.6 ± 0.06. No clinical cases of PEM were observed during the course of either study. The use of thiamin as a dietary additive to aide in the prevention of PEM in finishing lambs does not appear to be necessary under the conditions of this study.

Introduction

One of the challenges with use of ethanol co-products is the potential for high dietary S concentrations. High S diets can cause polioencephalomalacia (**PEM**) in ruminants (Gould, 1998). Inclusion of high proportions of distillers dried grains with solubles (**DDGS**) and other co-products in finishing rations for ruminants has been avoided; in part, due to potential problems with PEM as well as concerns about optimal animal performance and carcass characteristics. While the common dogma is that including DDGS at over 40% of dietary DM in beef cattle finishing diets will decrease performance, research indicates sheep can be fed higher concentrations of DDGS without affecting animal performance (Schauer et al., 2008). This provides an opportunity for increased utilization of DDGS in lamb finishing rations. Concerns remain regarding the effects of increased S concentrations in DDGS-based rations on incidence of PEM and if measures are available to reduce or prevent PEM. Thiamin supplementation is one proposed method of reducing or preventing PEM in ruminant animals (Brent and Bartley, 1984; McDowell, 2000; Olkowski et al., 1992). The involvement of thiamin in S-induced

PEM is unclear (Galyean and Rivera, 2003). The efficacy by which thiamin supplementation may prevent PEM in animals fed high dietary S is likely impacted by the PEM causative mechanisms (e.g. long-term thiamin deficiency or high hydrogen sulfide gas concentration). Further, the effective concentration of thiamin necessary to alleviate or prevent such cases of PEM requires additional investigation. A great portion of recent research efforts have focused on supplementing 150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin (Buckner et al., 2007; Huls et al., 2008; Schauer et al., 2008) to ruminants fed high amounts of DDGS. Our hypothesis was that providing increased dietary thiamin would decrease the incidence of PEM in lambs fed high S diets without affecting animal performance.

Procedures

All animal care and handling procedures were approved by the North Dakota State University Animal Care and Use Committee prior to the initiation of the research.

Study 1

Treatments and diets. The objective of this study was to determine the influence of thiamin concentration on ADG, DMI, G:F, carcass characteristics, and incidence of PEM in lambs. Two-hundred forty western white-faced Rambouillet wether and ewe lambs (BW ± SD; 32.5 ± 4.8 kg) were stratified by weight and sex and assigned randomly to 16 outdoor pens (15 lambs/pen). Pens were assigned randomly to one of four dietary treatments, with pen serving as experimental unit (n = 4 per treatment). Prior to initiation of this study lambs were vaccinated for clostridial disease (Convexin 8, Schering-Plough, Kenilworth, NJ) two weeks prior to weaning, at weaning, and again at the initiation of the study. Additionally, lambs were treated for coccidiosis at weaning for 10 d with Corid (9.6% Amprolium, Merial, Ltd., Duluth, GA). Lambs were adapted to an 80% concentrate diet

prior to initiation of study diets; previous diets are outlined in Table 2.1. None of these adaptation diets contained any DDGS.

Table 2.1. Diets fed to lambs in Study 1, prior to initiation of research diets (%, DM basis).

Ingredient	Weaning	2 wk Post-Wean	4 wk Post-Wean	6 wk Post-Wean
Creep Pellet[1]	100	50	25	--
Alfalfa	--	15	20	20
Corn	--	20	30	50
Barley	--	15	25	30

[1]Creep pellet contained: 16% CP, 3.5% crude fat, 12% crude fiber, 1% Ca, 0.55% P, 0.5% salt, 0.2 ppm Se, 2600 IU/lb vitamin A, 260 IU/lb vitamin D, 10 IU/lb vitamin E, and 50g/ton chlortetracycline.

Treatment diets were balanced to meet or exceed CP, NE, and Cu requirements (NRC, 2007). The dietary treatments were formulated to have minimum Ca to P ratio of 2:1, and ammonium chloride (0.5%, DM basis) was added to all diets to aid in the prevention of urinary calculi resulting from the elevated concentrations of P. The final finishing diet contained 60% DDGS (DM basis; Table 2.2) which resulted in a dietary S concentration of 0.73%. Concentrations of thiamin provided in our diets was based on previous research supplementing 150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin (Buckner et al., 2007; Huls et al., 2008; Schauer et al., 2008). The concentrations examined were created to demonstrate a response of supplementing no thiamin vs. graded concentrations up to 150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin to lambs fed large quantities of DDGS. Treatments were formed by increasing the amount of supplemental thiamin supplied and dietary concentrations of thiamin were formulated to provide the following amounts: no supplemental thiamin (**CON**), 2) 50 $mg \cdot hd^{-1} \cdot d^{-1}$ (**LOW**), 3) 100 $mg \cdot hd^{-1} \cdot d^{-1}$ (**MED**), or 4) 150 $mg \cdot hd^{-1} \cdot d^{-1}$ (**HIGH**) based on an estimated DMI of 1.36 kg.

Table 2.2. Ingredient and nutritional composition of diets fed in Study 1

	Diets[1]			
Item	CON	LOW	MED	HIGH
Ingredient, %	DM basis			
Alfalfa Hay	15.00	15.00	15.00	15.00
Corn	21.39	21.39	21.39	21.38
DDGS[2]	60.00	60.00	60.00	60.00
Ammonium Chloride	0.5	0.5	0.5	0.5
Limestone	2.25	2.25	2.25	2.25
Lasalocid[3]	0.085	0.085	0.085	0.085
TM package[4]	0.78	0.78	0.78	0.78
Copper Sulfate	0.002	0.002	0.002	0.002
Thiamin	0.00	0.004	0.007	0.011
Nutrient composition (analyzed)				
CP, %	23.7	23.3	23.4	23.6
NDF, %	28.8	30.7	28.8	31.4
ADF, %	10.5	10.5	10.9	11.1
S, %	0.74	0.69	0.71	0.72
Ca, %	1.33	1.59	1.17	1.08
P, %	0.68	0.69	0.70	0.72
Cu, mg/kg	11	12	9	11
Zn, mg/kg	71	67	63	59
Thiamin[5], mg/kg	5	55	105	155

[1] Diets were balanced to meet or exceed requirements set by (NRC, 2007). Treatments based on amount of supplemental thiamin provided: CON (no supplemental thiamin), LOW (50 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), MED (100 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), HIGH (150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), and HIGH+S (150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin with 0.87% S).

[2] Distillers dried grains with solubles.

[3] Lasalocid (Bovatec 68, Alpharma Inc., Fort Lee, NJ).

[4] Trace Mineral (TM) package contained: 11.7% Ca, 10.0% P, 14% salt, 0.1% K, 0.1% Mg, 20 mg/kg Co, 100 mg/kg I, 2,450 mg/kg Mn, 50 mg/kg Se, 2,700 mg/kg Zn, 300,000 IU/lb Vitamin A, 30,000 IU/lb Vitamin D_3, and 600 IU/lb Vitamin E.

[5] Formulated based on estimated feed intake of 1.36 $kg \cdot hd^{-1} \cdot d^{-1}$, amount of supplemental thiamin provided, and corrected for thiamin contained in remaining feed ingredients.

All feeds were ground to a common particle size through a 1.25 cm screen. Rations were ground and mixed in a grinder-mixer (GEHL Mix-All, Model 170, West Bend, WI) and provided ad-libitum via bulk feeders. Feeders were checked daily and cleaned of contaminated feed (fecal contamination, wet feed due to precipitation, etc.). Grab samples

of the ration were collected on d 0, 27, 56, 84, and 110 for laboratory analysis. Weight of the feed remaining in the feeder at the end of the study (feed refusal) was recorded for calculation of DMI and G:F. Water was sampled on d 56 and was analyzed by a commercial laboratory (Stearns DHIA, Sauk Centre, MN) for sulfate (141 mg/L).

Data collection procedures. Initial and final weights were the average of two consecutive d weights. Following the 110 d finishing period, lambs were transported (768 km) to Iowa Lamb Corp, Hawarden, IA for harvest and subsequent carcass data collection. Lambs with a live weight less than 50 kg 28 d prior to slaughter were not shipped, but were included in the feedlot performance data. Treatment distribution of lambs shipped for slaughter was as follows; 49, 48, 44, and 44 lambs on the CON, LOW, MED, and HIGH treatments, respectively.

Carcass data collected included HCW, leg score, conformation score, fat depth (over 12th rib), body wall thickness, ribeye area, flank streaking, quality and yield grades. Leg score, conformation score, and quality grade were scored on a scale of 1 to 15 (1 = cull; 15 = high prime). Flank streaking was assigned with scores of 100-199 = practically devoid, 200-299 = traces, 300-399 = slight, 400-499 = small, and 500-599 = modest. Percent boneless closely trimmed retail cuts (**%BCTRC**) was calculated using the following equation: [49.936 - (0.0848 × 2.205 × HCW, kg) - (4.376 × 0.3937 × fat depth, cm) - (3.53 × 0.3937 × body wall thickness, cm) + (2.456 × 0.155 × ribeye area, cm^2)] from Savell and Smith (2000). Carcass data was collected by trained personnel after a 24-h chill (temperature < 2°C, humidity near 100%).

Laboratory analyses. Feed and ort samples were dried using a forced-air oven (55°C; The Grieve Corporation, Round Lake, IL) for 48 h. Dried samples were ground

using a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 2 mm screen. Feed samples were analyzed for DM, ash, N; and Ca, Cu, and Zn (methods 4.1.06, 4.1.10, 4.2.10; and 968.08 respectively; AOAC, 1997). Concentrations of NDF (Robertson and Van Soest, 1991, as modified by Ankom Technology, Fairport, NY) and ADF (Goering and Van Soest, 1970, as modified by Ankom Technology) were determined using an Ankom 200 Fiber Analyzer (Ankom Technology) without sodium sulfite, with amylase, and without ash corrections as sequentials. Sulfur and thiamin were analyzed by inductively coupled argon plasma and AOAC procedure 942.23/HPLC, respectively, by a commercial laboratory (Midwest Laboratories, Omaha, NE).

Statistical analyses. Feedlot performance and carcass trait data were analyzed as a completely random design using the GLM procedures of SAS (SAS Inst. Inc., Cary, NY) with pen serving as the experimental unit. Carcass data was analyzed similarly, with missing data points from underweight lambs not included in the data set, but with pen still serving as experimental unit. The model included treatment. Linear and quadratic contrasts for increase concentration of thiamin supplementation were evaluated. Significance was declared at $P \leq 0.05$. In the case that a significant F-test was not observed but a contrast *P*-value was significant the difference will be discussed as a tendency.

Study 2

Treatments and diets. The objective of this study was to determine the influence of thiamin concentration on ADG, DMI, G:F, carcass characteristics, ruminal hydrogen sulfide gas concentration, and incidence of PEM in lambs. Fifty-five western white-faced Rambouillet wether lambs (38.4 ± 3.9 kg) were utilized in a completely random design to evaluate the influence of concentration of thiamin supplementation in lamb finishing diets

containing 60% DDGS (treatment diets were the same as previously described for Study 1). In the ethanol production process, equipment is often washed with chemicals such as sulfuric acid the resulting high sulfur wash water may be added to distiller's grains at various points in the production process. To test the influence of this additional sulfur, a fifth treatment was added in which dietary thiamin was supplemented at the HIGH concentration while dietary sulfur was increased from 0.73% to 0.87% (DM basis) by the addition of dilute sulfuric acid to DDGS (**HIGH+S)**. Lambs were given the same vaccination and coccidiosis treatment outlined in Study 1. Lambs were assigned to one of five treatment diets (Table 2.3) and individually penned and fed for 112 d. Treatment distributions were 12, 10, 10, 12, and 11 lambs for CON, LOW, MED, HIGH, and HIGH+S, respectively. The final finishing diet was balanced to contain 60% DDGS (DM basis), which resulted in a dietary S concentrations of either 0.73% (CON, LOW, MED, and HIGH) or 0.87% S (HIGH+S). Rations were mixed weekly and sub-sampled for laboratory analysis. Feed was offered daily at 0630. Content of feeders (feed refusals) were collected and weighed weekly. Feed and ort samples were analyzed for DM and nutrient content in the same manner as Study 1. Water samples were collected weekly and composited by equal volume (20 mL) and analyzed by a commercial laboratory (Stearns DHIA, Sauk Centre, MN) for sulfates (74.4 mg/L). Initial and final weights were the average of two consecutive d weights. Following the 112 d finishing period, lambs were harvested and carcass data collected at the NDSU Meats Laboratory. Carcass data was collected in the same manner as Study 1.

Ruminal gas cap sampling. To further evaluate the effects of supplemental thiamin concentration on ruminal gas cap H_2S concentration, twenty lambs were selected randomly

(4 per treatment) for use in collection of ruminal gas cap samples. Sampling for ruminal H_2S was conducted on 12 occasions beginning 6 d prior to initiation of treatment diets. Gas cap samples from these lambs were collected on d -6, -4, 0, 3, 7, 10, 14, 17, 21, 24, 28, and 31 of the feeding period. Gas samples were collected 4 h after feed was offered. On d 0, lambs began the dietary adaptation period which increased the concentrate concentration to 85% over 28 d (Table 2.4).

Table 2.3. Ingredient and nutritional composition of diets fed in Study 2

	Diets[1]				
Item	CON	LOW	MED	HIGH	HIGH+S
Ingredient, %	DM basis				
Alfalfa Hay	15.00	15.00	15.00	15.00	15.00
Corn	21.39	21.39	21.39	21.38	21.38
DDGS[2]	60.00	60.00	60.00	60.00	60.00
Ammonium Chloride	0.5	0.5	0.5	0.5	0.5
Limestone	2.25	2.25	2.25	2.25	2.25
Lasalocid[3]	0.085	0.085	0.085	0.085	0.085
TM package[4]	0.78	0.78	0.78	0.78	0.78
Copper Sulfate	0.002	0.002	0.002	0.002	0.002
Thiamin	0.00	0.004	0.007	0.011	0.011
Nutrient composition (analyzed)					
CP, %	23.3	23.6	23.4	22.7	23.5
NDF, %	33.2	33.0	34.5	32.8	34.4
ADF, %	10.8	11.0	11.6	11.6	11.3
S, %	0.76	0.69	0.75	0.71	0.87
Ca, %	1.55	1.42	1.65	1.66	1.77
P, %	0.79	0.81	0.92	0.91	0.87
Cu, mg/kg	9	6	6	27	21
Zn, mg/kg	195	190	186	184	188
Thiamin[5], mg/kg	5	55	105	155	155

[1] Diets were balanced to meet or exceed requirements set by (NRC, 2007). Treatments based on amount of supplemental thiamin provided: CON (no supplemental thiamin), LOW (50 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), MED (100 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), HIGH (150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), and HIGH+S (150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin with 0.87% S).

[2] Distillers dried grains with solubles.

[3] Lasalocid (Bovatec 68, Alpharma Inc., Fort Lee, NJ).

[4] Trace Mineral (TM) package contained: 11.7% Ca, 10.0% P, 14% salt, 0.1% K, 0.1% Mg, 20 mg/kg Co, 100 mg/kg I, 2,450 mg/kg Mn, 50 mg/kg Se, 2,700 mg/kg Zn, 300,000 IU/lb Vitamin A, 30,000 IU/lb Vitamin D_3, and 600 IU/lb Vitamin E.

[5] Formulated based on estimated feed intake of 1.36 $kg \cdot hd^{-1} \cdot d^{-1}$, amount of supplemental thiamin provided, and corrected for thiamin contained in remaining feed ingredients.

Table 2.4. Adaptation diets (%, DM basis) fed to lambs in Study 2

	diet					
	Arrival	Step 1	Step 2	Step 3	Step 4	Step 5
	d					
Ingredient	-6	0	7	14	21	28
Alfalfa Hay	46	46	46	35	25	15
Dry Rolled Corn	50.4	35.9	21.4	21.4	21.4	21.4
DDGS[1]	0	14.5	29	40	50	60
Supplement[2]	3.6	3.6	3.6	3.6	3.6	3.6

[1] Distillers dried grains with solubles.
[2] Supplement contained (% total diet): 0.5% ammonium chloride, 2.25% limestone, 0.085% Lasalocid (Bovatec 68), 0.78% trace mineral, 0.002% copper sulfate, and formulated to provide one of four concentrations of thiamin (0 50, 100, or 150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin).

Figure 2.1 depicts the relationship between dietary adaptation and ruminal H_2S sampling regimen. Ruminal fluid was also collected via rumenocentesis at the same time ruminal gas cap samples were collected for determination of ruminal pH.

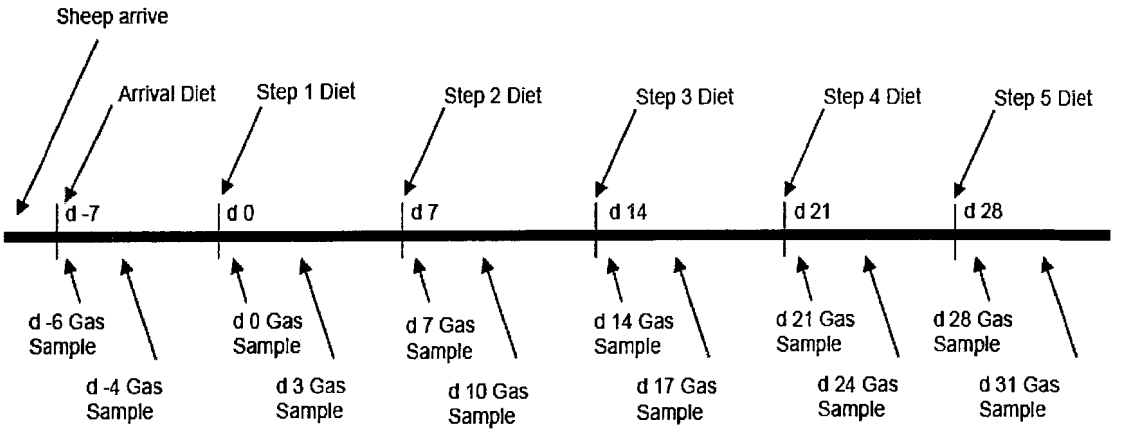

Figure 2.1. Dietary adaptation and gas sampling regimen applied to lambs in Study 2.

Procedures for ruminal gas cap sampling were adapted from those of Gould et al. (1997). In order to obtain ruminal gas cap samples, wool was sheared from a 15 cm by 15 cm area of the animals left side immediately posterior to the 13^{th} rib. Shearing was done

with surgical clippers with care taken to remove all wool. After shearing, this area was scrubbed and disinfected with alternating isopropyl alcohol and Betadine scrubs. In order to accomplish multiple samples while maintaining the integrity of the rumen gas, two separate portions of the sampling apparatus were developed (Figure 2.2). The first portion included the 7.6 cm 12 gauge needle which was connected to a 20 cm (4.75 mm diam.) tubing (Tygon ®, S-50-HL Class VI) via a Luer-lock connection. The second portion of the sampling apparatus included a 140 mL catheter tip syringe (Monoject, Sherwood Medical, Ballymoney, N. Ireland) which was connected to an 8 cm (4.75 mm diam.) portion of tubing via Luer-lock connection. The two portions were then connected or disconnected through Luer-lock connections with ratchet tubing clamps utilized on both sides of the Luer-lock connectors.

After the needle was introduced thru the skin and into the rumen gas cap a 120 mL sample (approx.) of ruminal gas was drawn into the syringe. The first of two syringes was then disconnected and a second filled in the same manner. Hydrogen sulfide gas detector tubes (Gastec©, Kanawaga Japan) were connected to a volumetric gas sampling pump and a volume (100 mL) was drawn through the detector tube to acquire a measurement of ruminal gas cap H_2S. At each sampling point duplicate measurements were taken from each lamb and the average of the two samples was used for any calculations. If the detector tube failed to reach 100 ppm hydrogen sulfide (the lowest detectable concentration recommended by the manufacture) the reading was treated as a zero.

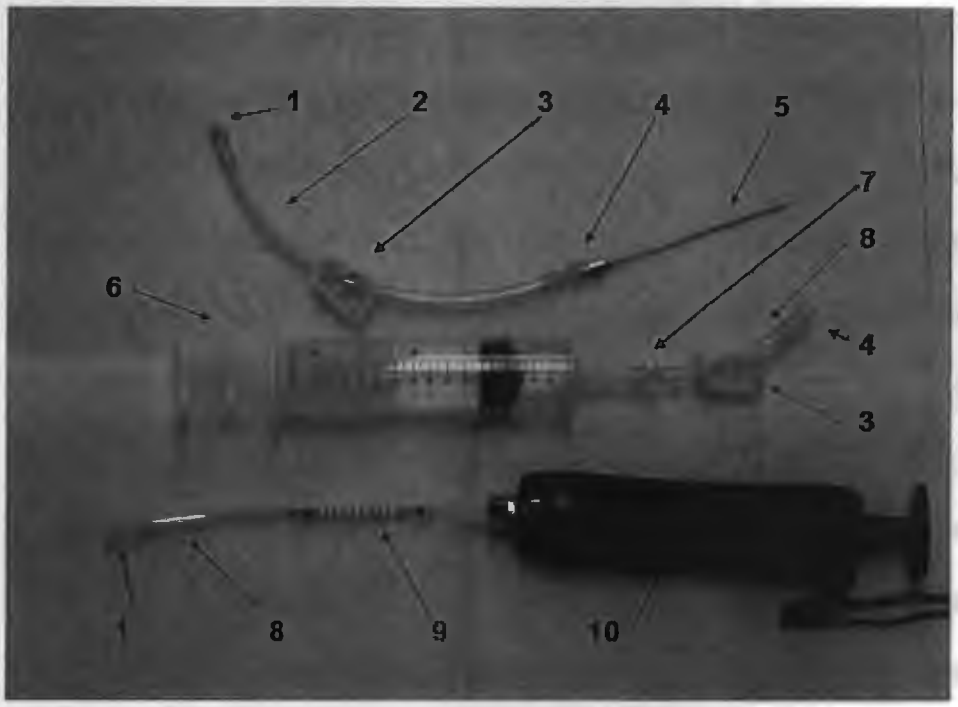

Figure 2.2. Sampling apparatus. Parts required for assembly: 1) male Luer-lock connector, 2) 20 cm (4.75mm diam.) tubing (Tygon ®, S-50-HL Class VI), 3) ratchet tubing clamp, 4) female Luer-lock connector, 5) 7.6 cm 12 gauge needle, 6) 140 mL catheter tip syringe (Monoject, Sherwood Medical, Ballymoney, N. Ireland), 7) Oversized male Luer-lock connector, 8) 8 cm (4.75 mm diam.) tubing (Tygon ®, S-50-HL Class VI), 9) Hydrogen sulfide gas detector tube (Gastec©, Kanawaga, Japan), and 10) Gas sampling pump (Gastec Model GV-100, Gastec©, Kanawaga, Japan).

Following gas and fluid sampling, the needle was removed and the sampling site was sprayed with a 10% Iodine solution. Ruminal hydrogen sulfide concentrations were converted from parts per million H_2S to grams per cubic meter through the following equation: [(Hydrogen sulfide (ppm) × 139.06)/1000000] assuming standard temperature and pressure values.

Pathological examination of brain tissue. Brain tissue was collected for histological observation of microscopic lesions associated with polioencephalomalacia at slaughter. Lambs were stunned using a captive bolt, in a manner which caused cervical dislocation. This was done to decrease any tissue damage to the cortex and cerebellum

regions of the brain. Lamb heads were then removed and split laterally with a band saw (Hobart Industries, Troy, OH) to remove all intact brain tissue. Tissue was placed in 10% neutral buffered formalin for a minimum of 3 d for fixation. After fixation, each brain was sampled for histolopathologic analysis in a manner similar to Carson (1990). For each animal, sections of cerebral cortex, cerebellum, hippocampus and brainstem were processed with 10% NBF, 70% alcohol, xylene and paraffin using automated procedures (Sakura VIP Tissue Processor). Paraffinized tissues were then sectioned to 5 microns and stained (Sakura, Tissue-Tek, H+E stainer) using xylene, alcohol, distilled water, hematoxylin, bluing agent and eosin. Tissues were examined for features associated with PEM by a board certified veterinary pathologist who was blind to treatment assignments.

Statistical analyses. Lamb performance and carcass trait data were analyzed as a completely randomized design using the GLM procedures of SAS with lamb serving as the experimental unit. The model included treatment. Linear and quadratic contrasts were used to evaluate the effect of increasing concentration of thiamin supplementation. In addition, a contrast was also utilized for a direct comparison of HIGH vs. HIGH+S treatments in order to evaluate the effect of increased S concentration with the high concentration of thiamin. Significance was declared at $P \leq 0.05$. In the case that a significant F-test was not observed but a contrast *P*-value was significant the difference will be discussed as a tendency. Hydrogen sulfide gas and pH data were analyzed utilizing the repeated measures analysis in the Mixed Procedures of SAS with *P*-values ≤ 0.05 considered significant. Treatment, day, and the treatment by day interaction were all evaluated. The covariate structure used was autoregressive [AR(1)]. Other structures were tested; however autoregressive was the best fit.

Results

Study 1

Results for feedlot lamb performance and carcass quality are reported in Table 2.5. There was a tendency for quadratic ($P = 0.08$) increases in final BW; specifically the CON, LOW, and MED treatment lambs had heavier final BW than the lambs fed the HIGH concentration of thiamin. Average daily gain exhibited a similar quadratic response ($P = 0.04$) with the CON, LOW, and MED treatment groups gaining weight at a faster rate than the HIGH treatment group. Dry matter intake decreased quadratically ($P < 0.01$) with increasing concentration of thiamin supplementation. Gain-to-feed ratio tended to differ linearly ($P = 0.08$) with the MED fed lambs having greater DMI which in turn resulted in decreased G:F.

Mortality was not affected ($P = 0.43$) by concentration of supplemental thiamin and averaged 0.42% across all treatments. Hot carcass weight (HCW) tended to decrease quadratically ($P = 0.05$), while leg score had a quadratic tendency ($P = 0.06$) for a lower score with increased thiamin supplementation. Fat depth, body wall thickness, ribeye area, flank streaking, quality grade, and yield grade were all unaffected ($P > 0.16$) by concentration of supplemental thiamin.

Study 2

Performance measures. There were no differences ($P \geq 0.46$) in initial BW, final BW, ADG, DMI, leg score, carcass conformation, fat depth, body wall thickness, ribeye area, yield grade, or % boneless closely trimmed retail cuts (Table 2.6) due to thiamin concentration. There was an overall treatment effect ($P = 0.03$) for flank streaking. This was largely due to the difference in flank streaking between the HIGH and the HIGH+S

groups (325.0 vs. 481.8, respectively for HIGH and HIGH+S; $P = 0.002$). The increase in flank streaking resulted in a difference ($P = 0.02$) in quality grade between HIGH and HIGH+S treatment groups. No incidences of morbidity or mortality occurred.

Pathology of brain tissue samples. Gross examination of whole brains did not show classic changes associated with PEM such as yellowing and softening of the cerebral cortex and flattening of gyri. Further, microscopic features consistent with PEM were not observed. Extravasated red blood cells were frequently present within the meningial spaces as well as around blood vessels in the thalamus and cerebellum; however this appeared to be a result of the method of euthanasia rather than representative of pathologic condition.

Table 2.5. Influence of thiamin supplementation on performance and carcass characteristics of lambs in Study 1

	Treatment[1]						*P*-value[3]	
Item	CON	LOW	MED	HIGH	SEM[2]	*P*-value	Linear	Quad
Initial Wt, kg	32.6	32.6	32.5	32.6	0.15	0.94	0.73	0.63
Final Wt, kg	62.3	62.8	62.5	60.5	0.65	0.10	0.07	0.08
ADG, kg/d	0.268	0.274	0.272	0.253	0.005	0.08	0.09	0.04
Intake, $kg \cdot hd^{-1} \cdot d^{-1}$	1.77	1.78	1.98	1.74	0.04	0.001	0.49	0.004
G:F, kg gain/kg DMI	0.15	0.15	0.14	0.15	0.004	0.05	0.08	0.57
Mortality, %	1.67	0	0	0	0.83	0.43	0.20	0.34
HCW, kg	31.4	32.1	31.7	30.9	0.37	0.18	0.35	0.05
Leg Score[4]	11.32	11.48	11.60	11.05	0.17	0.16	0.36	0.06
Conformation Score	11.50	11.42	11.57	11.23	0.09	0.09	0.12	0.17
Fat Depth[5], cm	0.79	0.86	0.76	0.84	0.05	0.59	0.96	0.96
Body Wall Thick, cm	2.72	2.99	2.54	2.67	0.10	0.32	0.39	0.83
Ribeye Area, cm^2	15.6	15.5	15.7	15.7	0.39	0.98	0.77	0.92
Flank Streaking[6]	336.92	340.25	353.33	336.36	6.74	0.29	0.71	0.16
Quality Grade	11.34	11.33	11.47	11.18	0.08	0.17	0.36	0.13
Yield Grade[7]	3.48	3.75	3.42	3.66	0.18	0.55	0.82	0.94
BCTRC[8], %	44.66	44.33	45.01	46.81	0.21	0.18	0.24	0.75

[1] Treatments based on amount of supplemental thiamin provided: CON (no supplemental thiamin), LOW (50 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), MED (100 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), and HIGH (150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin).

[2] Standard Error of Mean; n = 4.

[3] *P*-value for linear and quadratic effects of increasing concentration of thiamin supplementation.

[4] Leg score, conformation score, and quality grade: 1 = cull to 15 = high prime.

[5] Adjusted fat depth and yield grades.

[6] Flank streaking: 100-199 = practically devoid; 200-299 = traces; 300-399 = slight; 400-499 = small; 500-599 = modest.

[7] Yield Grade = 0.4 + (10 x adjusted fat depth).

[8] Boneless closely trimmed retail cuts (%) [49.936 - (0.0848 × 2.205 × HCW, kg) - (4.376 × 0.3937 × fat depth, cm) - (3.53 × 0.3937 × body wall thickness, cm) + (2.456 × 0.155 × ribeye area, cm^2)].

Table 2.6. Influence of thiamin supplementation and added sulfur on performance and carcass characteristics of lambs in Study 2

	Treatment[1]							*P*-value[3]		
Item	CON	LOW	MED	HIGH	HIGH+S	SEM[2]	*P*-value	Linear	Quad	H vs HS
Initial Wt, kg	38.5	38.7	38.4	38.1	38.8	1.30	0.99	0.79	0.85	0.70
Final Wt, kg	59.7	61.0	58.1	60.7	60.9	1.72	0.74	0.99	0.69	0.94
ADG, kg/d	0.189	0.199	0.175	0.201	0.193	0.011	0.48	0.76	0.46	0.77
Intake, $kg \cdot hd^{-1} \cdot d^{-1}$	1.30	1.30	1.24	1.27	1.30	0.05	0.86	0.44	0.70	0.68
G:F, kg gain/ kg DMI	0.15	0.15	0.14	0.16	0.15	0.005	0.17	0.15	0.32	0.20
HCW, kg	30.6	31.8	29.2	29.3	31.5	0.97	0.40	0.60	0.90	0.56
Leg Score[4]	11.08	11.10	10.80	11.00	11.18	0.22	0.78	0.55	0.67	0.54
Conformation Score	10.75	10.90	10.70	10.75	11.09	0.19	0.56	0.80	0.79	0.18
Fat Depth[5], cm	0.79	0.69	0.69	0.74	0.86	0.10	0.60	0.71	0.35	0.35
Body Wall Thick, cm	2.62	2.44	2.40	2.46	2.67	0.13	0.46	0.37	0.36	0.21
Ribeye Area, cm^2	14.4	14.1	13.7	14.9	14.3	0.58	0.70	0.62	0.21	0.45
Flank Streaking[6]	391.67	370.00	350.00	325.00	481.82	37.25	0.03	0.16	0.96	0.002
Quality Grade	11.25	11.10	11.10	11.00	11.73	0.22	0.12	0.41	0.91	0.02
Yield Grade[7]	3.48	3.10	3.05	3.31	3.76	0.36	0.60	0.71	0.35	0.35
BCTRC[8], %	44.68	44.74	45.16	45.14	44.26	0.47	0.59	0.35	0.93	0.16

[1]Treatments based on amount of supplemental thiamin provided: CON (no supplemental thiamin), LOW (50 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), MED (100 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), HIGH (150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), and HIGH+S (150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin with 0.87% S).

[2]Standard Error of Mean; n = 12, 10, 10, 12, and 11 for CON, LOW, MED, HIGH, and HIGH+S respectively.

[3]*P*-value for linear and quadratic effects of increasing concentration of thiamin supplementation.

[4]Leg score, conformation score, and quality grade: 1 = cull to 15 = high prime.

[5]Adjusted fat depth and yield grades.

[6]Flank streaking: 100-199 = practically devoid; 200-299 = traces; 300-399 = slight; 400-499 = small; 500-599 = modest.

[7]Yield Grade = 0.4 + (10 x adjusted fat depth).

[8] Boneless closely trimmed retail cuts (%) [49.936 - (0.0848 × 2.205 × HCW, kg) - (4.376 × 0.3937 × fat depth, cm) - (3.53 × 0.3937 × body wall thickness, cm) + (2.456 × 0.155 × ribeye area, cm^2)].

Ruminal H_2S concentration and ruminal pH. There was a treatment by day interaction ($P < 0.001$) for ruminal H_2S concentrations. No differences in H_2S concentration ($P > 0.05$; Figure 2.3) among treatments were apparent until d 10, at which point lambs fed LOW had lower ruminal H_2S concentrations than all other treatments. Lambs fed HIGH had the greatest concentrations of H_2S on d 31 (1.07 g H_2S/m^3; $P < 0.009$). At this time point, lambs fed HIGH+S had lower ($P < 0.01$) concentrations of H_2S than lambs fed HIGH. Ruminal pH did not differ ($P = 0.13$) and averaged 5.6 ± 0.06 across all treatments.

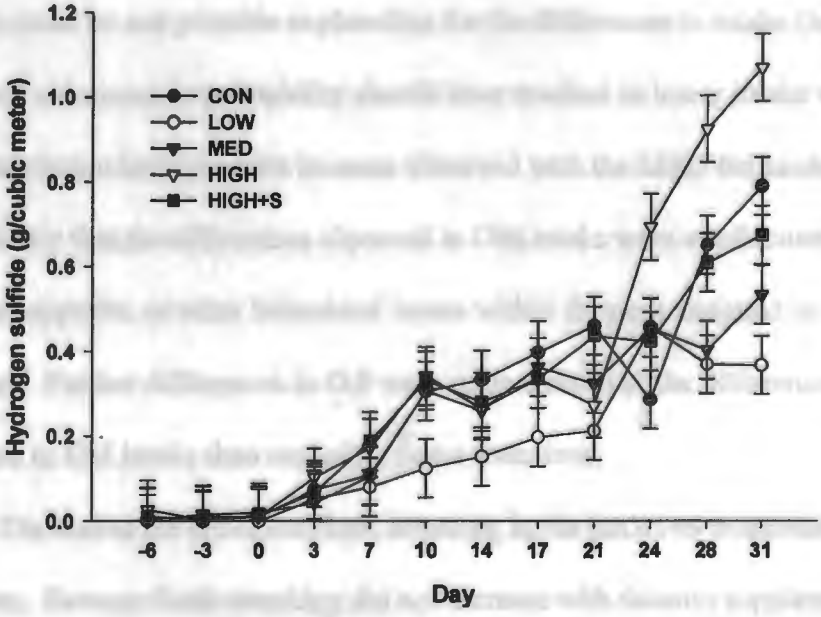

Figure 2.3. Change in hydrogen sulfide gas concentration [g/m^3], due to increasing dietary thiamin and sulfur concentrations, in lambs over adaptation from a medium concentrate corn and alfalfa hay based diet to a 60% DDGS finishing ration. Treatments were: no supplemental thiamin (**CON**), 2) 50 $mg \cdot hd^{-1} \cdot d^{-1}$ (**LOW**), 3) 100 $mg \cdot hd^{-1} \cdot d^{-1}$ (**MED**), 4) 150 $mg \cdot hd^{-1} \cdot d^{-1}$ (**HIGH**), and 5) 150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin with increased S (**HIGH+S**) thiamin levels were based on an estimated DMI of 1.36 kg. *P*-values: treatment $P = 0.03$, day $P < 0.001$, treatment x day $P < 0.001$. Concentrations of hydrogen sulfide gas measured via rumenocentesis on Hydrogen sulfide detector tubes (Gastec©, Kanawaga, Japan).

Discussion

The decrease in final weight with increasing concentration of thiamin in Study 1 was an unexpected result. Given that excess thiamin is cleared by the kidneys (McDowell, 2000) and that intake of upwards of 1000 times requirement are thought to be safe (NRC, 1987), it is difficult to attribute the decreased performance to thiamin toxicity at the concentrations fed in the present study. The cause of the lower ADG could be attributed to the decreased feed intake observed in the HIGH fed lambs; however feed conversion (G:F) was the same for CON and HIGH fed lambs.

Palatability differences due to the sulfurous odor and bitter taste associated with thiamin could be one possible explanation for the differences in intake (McDowell, 2000). However, a decrease in palatability should have resulted in lower intake with increasing supplementation instead of the increase observed with the MED fed lambs in Study 1. It is just as likely that the differences observed in DM intake were a reflection of competition, increased appetite, or other behavioral issues within the pens assigned to that particular treatment. Further differences in G:F were more a result of the differences previously described in DM intake than any other factor examined.

The reason for increased flank streaking in the HIGH+S treatment in Study 2 is unknown. Because flank streaking did not increase with thiamin supplementation it is highly unlikely this response is due to thiamin supplementation. Previous work (Smith et al., 1964) reported that lambs receiving 0.43% S had carcasses which graded higher compared to lambs fed either 0.13 or 1.3% S. Schauer et al., (2008) also reported lambs having higher flank streaking when feeding increased concentrations of DDGS, attributing the increases in carcass quality to increases in energy density of the diet.

No differences in mortality were observed in either study due to concentration of thiamin supplementation. There was one case of mortality in Study 1; the cause of death, as determined by a veterinarian, was chronic respiratory illness. No occurrences of PEM were observed during either of these studies; even with dietary S concentrations (0.73% S DM basis) more than twice the recommended maximum tolerable concentration (0.3% S for high concentrate diets; NRC, 2005). Contrary to the present studies, Krasicka et al. (1999) reported that all lambs fed a low fiber-high starch diet containing 0.72% S died from PEM after 12 weeks. Olkowski et al. (1992) reported that outward clinical signs of PEM in lambs fed high sulfur diets (0.63%) were prevented by supplementing thiamin. However, microscopic lesions were not totally prevented (Olkowski et al., 1992). The present studies are contradictory to previous research; in that, no outward signs or microscopic lesions associated with PEM were observed even without supplemental thiamin. Loneragan et al. (2005) hypothesized that the therapeutic effects of thiamin in PEM-affected animals may be attributed to either an increased requirement for thiamin or a beneficial effect of thiamin on impaired brains. The present research discounts the proposed increased requirement; at least in feedlot lambs fed DDGS as the major S source; however, we cannot support or dismiss the second theory, relating to the beneficial effect of thiamin on impaired brains, as no cases of PEM occurred in our study. Our data suggests that PEM could not be induced in lambs fed diets containing > 0.7% S; which indicates that the threshold S concentration which causes PEM may be greater in lambs than previously thought or that disposition of S, including its chemical form in feed and water sources (e.g. sulfate, S-containing amino acids), the fate of S in the digestive tract (especially as it relates to fermentation and digestion), and route of excretion for excess S

(e.g. H_2S excretion during eructation, S excretion in urine or feces) should be investigated more fully.

Further links between sulfur induced PEM and ruminal pH change have been explored by Gould (1998), who concluded that in diets with S concentrations exceeding 0.3 %, the combination of dietary S concentration, ruminal sulfide production, and increased thiaminase production may increase incidence of PEM. Alves de Oliveria et al. (1996) reported decreased ruminal pH did not decrease microbial production of thiamin; however, the decrease in rumen pH does favor thiaminase producing bacteria (Morgan and Lawson, 1974; Boyd and Walton, 1977; Thomas et al., 1987). Other researchers have examined ruminal H_2S concentration in cattle. Loneragan et al. (2005) reported that feedlot steers receiving water containing 2,360 mg sulfate/L had peak ruminal H_2S gas concentrations between 5500 and 6000 ppm. Likewise, Niles et al. (2002) report steers consuming a corn gluten feed-based ration with 0.701% S had ruminal gas cap H_2S concentrations of 18,642 ppm on d 28 of their study.

A review of literature reporting the amount of S fed to ruminants in corn byproduct-based rations simply confirms the inconsistencies in the amount of S required to cause neurological problems such as PEM. Similar to the present studies, Schauer et al. (2008) fed lambs a finishing diet which contained 0, 20, 40, or 60% DDGS. No differences in animal performance were reported; further, no incidences of PEM were noted when lambs were fed the 60% DDGS diet which contained 0.55% S (DM basis). Contrary to the present studies, (Niles et al., 2002) reported that 10 of 14 calves fed a corn gluten feed-based diet exhibited PEM; those calves affected were fed diets that contained either 0.554 or 0.701% S (DM basis). Both authors reported water sulfate values; the water consumed

by the lambs (Schauer et al., 2008) contained 141 mg sulfate/L, while the water consumed by the steers (Niles et al., 2002) contained 56 mg sulfate/L. Unfortunately, Niles et al. (2002) did not report how much, if any, supplemental thiamin was provided to the steers in their study; however, Schauer et al. (2008) reported lambs in their study received 142 $mg \cdot hd^{-1} d^{-1}$ of supplemental thiamin. Huls et al. (2008) reported that steers fed 50% modified distillers grains plus solubles (DM basis) and supplemented with 150 $mg \cdot hd^{-1} d^{-1}$ thiamin performed similarly to steers fed a high-moisture corn and dry-rolled corn-based diet. Contrary to these results, Buckner et al. (2007) discontinued feeding a treatment diet which contained 50% DDGS (DM basis) when multiple steers exhibited PEM while receiving 150 $mg \cdot hd^{-1} d^{-1}$ thiamin.

Sulfur from water sources has also been implicated as a cause of PEM in ruminants. Ward and Paterson (2004) evaluated thiamin supplementation as a method of preventing PEM in steers consuming high sulfate (4000 mg/L) water. Two steers on high sulfate water and one steer from high sulfate water supplemented with 1000 $mg \cdot hd^{-1} d^{-1}$ thiamin died; however, only one of the cases from the unsupplemented group was confirmed to have died from PEM. Although no incidences of PEM occurred, Loneragan et al., (2001) reported consuming water with increasing sulfate concentrations negatively impacted steer performance and carcass characteristics. However, this decrease in performance was not observed in lambs fed DDGS diets containing increasing amount of S (Schauer et al, 2008). It is possible that cattle and sheep have different tolerances for excess S or different tolerances for ruminal H_2S. Consequently additional research is needed to confirm whether or not sheep are a good model for cattle for this type of research.

Further research is warranted to determine if the NRC (2005) maximum tolerable level of S fully describes how various forms of S are processed within the ruminant animal and under what conditions 0.3% S causes PEM. At a minimum, the current literature illustrates the need for additional research to further determine the interactive affects of S, thiamin supplementation, and dietary grain concentration in finishing rations, and the effect they collectively have on the incidence of PEM. In addition, research is needed to evaluate these practices in cattle and to determine the effect of the form of S in the feed and water as well as its fate in the animal (absorption, retention, and excretion).

Literature Cited

Alves de Oliveria, L., D. Jean-Blain, A. Durix, S. Komisarczuk-Bony, and C. Durier. 1996. Use of a semi-continuous culture system (RUSITEC) to study the effect of pH on microbial metabolism of thiamin (Vitamin B1). Arch. Anim. Nutr. 49:193-202.

AOAC. 1997. Official Methods of Analysis. 15th ed. Assoc. of Offic. Anal. Chem. Arlington, VA.

Boyd, J. W., and J. R. Walton. 1977. Cerebrocortical necrosis in ruminants: An attempt to identify the source of thiaminase in affected animals. J. Comp. Pathol. 87:581-589.

Brent, B. E., and E. E. Bartley. 1984. Thiamin and niacin in the rumen. J. Anim. Sci. 59:813-822.

Buckner, C. D., T. L. Mader, G. E. Erickson, S. L. Colgen, K. K. Karges, and M. L. Gibson. 2007. Optimum concentrations of dry distillers grains with solubles for finishing beef steers. 2007 Nebraska Beef Report. MP90:36-38.

Carson, F. L. 1990. Histology: A self-instructional text. ASCP Press. Chicago, IL.

Galyean, M. L., and J. D. Rivera. 2003. Nutritionally related disorders affecting feedlot cattle. Can. J. Anim. Sci. 83:13-20.

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.

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

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

Huls, T. J., M. K. Luebbe, G. E. Erickson, and T. J. Klopfenstein. 2008. Effect of inclusion level of modified distillers grains plus solubles in finishing steers. 2008 Nebraska Beef Report. MP91:41-42.

Krasicka, B, M. A., Gralak, B. Sieranska, and G. Kulasek. 1999. The influence of dietary sulphur loading on metabolism and health in young sheep fed low fibre and high starch diet. Reprod. Nutr. Dev. 39:625-636.

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.

Loneragan, G, D. Gould, J. Wagner, F. Garry, and M. Thoren. 2005. The magnitude and patterns of ruminal hydrogen sulfide production, blood thiamin concentration, and mean pulmonary arterial pressure in feedlot steers consuming water of different sulfate concentrations. Bov. Pract. 39:16-22.

McDowell, L. R. 2000. Vitamins in animal and human nutrition. 2nd Edition. Iowa State University Press. Ames, IA.

Morgan, K. T., and G. H. Lawson. 1974. Thiaminase type 1-producing bacilli and ovine polioencephalomalacia. Vet. Rec. 95:361-363.

National Research Council. 1987. Vitamin tolerances or animals. National Academy of Science-National Research Council, Washington, D.C.

National Research Council. 2005. Mineral tolerances of animals, Second revised edition. The National Academies Press. Washington, D.C.

National Research Council. 2007. Nutrient requirements of small ruminants. The National Academies Press. Washington, D.C.

Niles, G. A., S. Morgan, W. C. Edwards, and D. Lalman. 2002. Effects of dietary sulfur concentrations on the incidence and pathology of polioencephalomalacia in weaned beef calves. Vet. Human Toxicol. 44(2): 70-72.

Olkowski, A. A., S. R. Gooneratne, C. G. Rousseaux, and D. A. Christensen. 1992. Role of thiamine status in sulphur induced polioencephalomalacia in sheep.3.332 Res. Vet. Sci. 52:78.

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

Savell, J. W., and G. C. Smith. 2000. Laboratory manual for meat science. 7th Edition. American Press. Boston, MA.

Schauer, C. S., M. M. Stamm, T. D. Maddock, and P. B. Berg. 2008. Feeding 60% of lamb finishing rations as dried distillers grains with solubles results in acceptable performance and carcass quality. Sheep and Goat Res. J. 23:15-19.

Smith, G. C., M. W. Galgen, and M. Weller. 1964. Effect of elemental sulfur on lamb performance and carcass quality. J. Anim. Sci. 23:892. (Abst.).

Thomas, K. W., D. L. Turner, and E. M. Spicer. 1987. Thiamine, thiaminase and transketolase levels in goats with and without polioencephalomalacia. Aust. Vet. J. 64:126-127.

Ward, E. H., and H. H. Patterson. 2004. Effects of thiamin supplementation on performance and health of growing steers consuming high sulfate water. Proc. West. Sec. Am. Soc. Anim. Sci. 55:375-378.

CHAPTER III. SULFUR BALANCE AND RUMINAL HYDROGEN SULFIDE CONCENTRATIONS IN LAMBS FED INCREASING CONCENTRATIONS OF DISTILLERS DRIED GRAINS PLUS SOLUBLES

Abstract

Feeding increased concentrations of distillers dried grains with solubles (**DDGS**) has been implicated as a cause of S toxicity in ruminants. Elucidating the mechanism by which dietary S causes polioencephalomalacia (**PEM**) is of importance to the livestock feeding industry. Our hypothesis was that lambs fed increased concentrations of DDGS will increase S excretion to avoid toxicity. Further, we hypothesized that feeding increased concentrations of DDGS will increase ruminal H_2S concentrations. The objective of this study was to evaluate the effects of increasing dietary concentration of DDGS on S balance and ruminal hydrogen sulfide gas concentrations in lambs. Sixteen western white-faced Rambouillet wether lambs (36.7 ± 2.3 kg) were utilized in a completely random design to evaluate this objective. Treatments were based on increasing concentrations of DDGS in the final finishing diet and included: 1) 0% DDGS, 2) 20% DDGS, 3) 40% DDGS, and 4) 60% DDGS. Ruminal hydrogen sulfide concentrations were measured weekly via rumen puncture. Ruminal gas was collected as lambs were adapted from a medium concentrate diet to their respective finishing diets. Lambs were placed in metabolism crates for a 10 d adaptation period. Following adaptation to metabolism crates, lambs were fitted with fecal collection bags. Feed, water, feces, and urine were collected, weighed, and sub-sampled daily. There was a day by treatment interaction ($P < 0.001$) for ruminal H_2S concentration. Hydrogen sulfide concentrations did not differ ($P \geq 0.06$) until d 14 when lambs fed 60% DDGS had greater hydrogen sulfide concentrations (0.23 ± 0.039 g/m^3; $P \leq 0.006$) than all other treatments. Peak H_2S concentrations occurred on d 14 (0% DDGS; 0.01 g/m^3), 21

(20% DDGS; 0.08 g/m^3), and 28 (60% DDGS; 0.49 g/m^3). Dietary DDGS inclusion did not affect DMI (1.37 ± 0.07 kg·hd^{-1}·d^{-1}; $P = 0.25$). Sulfur intake from feed and water, as well as S excretion in feces and urine increased linearly ($P \leq 0.009$) with increasing DDGS inclusion. Sulfur balance increased linearly ($P = 0.02$) with increasing inclusion of DDGS in finishing diets. Increasing concentration of DDGS in the diet increased S intake, excretion, and H_2S concentrations but did not result in the occurrence of PEM. This research suggests that substantial amounts of S in DDGS are excreted by the ruminant animal.

Introduction

Feeding increased concentrations of distillers dried grains with solubles (**DDGS**) to ruminants has been avoided due to risks of S toxicity and concerns about animal performance. High S diets can cause polioencephalomalacia (**PEM**) in ruminants (Gould, 1998). However, research has demonstrated that lambs fed 60% DDGS did not develop PEM (Neville et al., 2010a) and performed similar to those fed lesser concentrations of DDGS (Schauer et al., 2008). Schauer et al. (2008) and Neville et al. (2010a) provide an opportunity for increased utilization of DDGS in lamb finishing rations. However, this research stands in contrast to other findings in lambs (Low et al., 1996) and beef cattle (Zinn et al., 1999; Lamm et al., 2010) which characterize dietary S as a primary cause of PEM. The recommendations outlined by NRC (2005) list 0.3% S as the maximum tolerable level for ruminants consuming high concentrate diets. Elucidating the mechanism by which lambs fed 0.7% S did not develop PEM (Neville et al., 2010a) is of importance to the livestock feeding industry and could potentially increase the utilization of DDGS in lamb finishing rations.

Feed and water are the two sources of dietary S. Sulfur is primarily excreted as sulfate in the urine or as organic S in feces (Underwood and Suttle, 1999) or eructated as hydrogen sulfide (H_2S; Dougherty et al., 1965). Sulfur balance research played a key role in determining the S requirements and will play a role in determination of S toxicity in ruminants. Research exploring animal adaptation to tolerate nutrient concentrations in excess of requirement is warranted.

We hypothesized lambs fed increased concentrations of DDGS would increase excretion of S to avoid toxicity. Further, we hypothesized that feeding increased concentrations of DDGS would increase ruminal H_2S concentrations. The objective of this study was to evaluate the effects of increasing dietary concentration of DDGS on S balance and ruminal H_2S gas concentrations in lambs.

Materials and Methods

All animal care and handling procedures were approved by the North Dakota State University Animal Care and Use Committee prior to the initiation of the research.

Animals and Treatments

Sixteen western white-faced Rambouillet wether lambs (36.7 ± 2.3 kg) were utilized in a completely random design to evaluate the effects of increasing dietary concentration of DDGS on S balance and ruminal H_2S gas concentrations in lambs. Treatments were based on increasing concentrations of DDGS in the final finishing diet and included: 1) 0% DDGS, 2) 20% DDGS, 3) 40% DDGS, and 4) 60% DDGS. Prior to initiation of this study, lambs were vaccinated for clostridial disease (Convexin 8, Schering-Plough, Kenilworth, NJ) two weeks prior to weaning, at weaning, and again at the initiation of the study. Additionally, lambs were treated for coccidiosis beginning at

weaning for 10 d with Corid (9.6% Amprolium, Merial, Ltd., Duluth, GA). Lambs were maintained on a medium concentrate diet prior to initiation of study diets. The diets fed from weaning to the initiation of the study are presented in Table 3.1.

Table 3.1. Diets fed to lambs prior to initiation of research diets (%, DM basis)

Ingredient	Weaning	2 wk Post-Wean	4 wk Post-Wean	6 wk Post-Wean
Creep Pellet[1]	100	50	25	--
Alfalfa	--	15	20	20
Corn	--	20	30	50
Barley	--	15	25	30

[1]Creep pellet contained: 16% CP, 3.5% crude fat, 12% crude fiber, 1% Ca, 0.55% P, 0.5% salt, 0.2 ppm Se, 2600 IU/lb vitamin A, 260 IU/lb vitamin D, 10 IU/lb vitamin E, and 50g/ton chlortetracycline.

The adaptation diets did not contain DDGS. Treatment diets were formulated to meet or exceed CP and Cu requirements; NE level in the diet was formulated for a lamb gaining 400 g/d (NRC, 2007; Table 3.2). The dietary treatments were formulated to provide minimum Ca to P ratio of 1.5:1, and ammonium chloride (0.5%, DM basis) was added to all diets to aid in the prevention of urinary calculi. Thiamin was included in all diets at a concentration which would provide 150 $mg \cdot hd^{-1} \cdot d^{-1}$ based on 1.36 kg estimated DMI.

Ruminal Hydrogen Sulfide Sampling

Ruminal H_2S gas concentrations were measured weekly via rumen puncture as lambs were adapted from a medium concentrate diet to their respective high concentrate finishing rations. On d 0, lambs began the dietary adaptation period which increased the concentrate portion of the diet to 85% over 28 d (Table 3.3). Hydrogen sulfide measurements were collected on d -7, 0, 7, 14, 21, 28, and 35 of the adaptation period. Ruminal fluid was also collected via rumenocentesis at the same time ruminal gas cap samples were collected. Ruminal pH was determined immediately with a combination

electrode (model 2000 pH/temperature meter; VWR Scientific Products, West Chester, PA).

Table 3.2. Ingredient and nutritional composition of diets fed to lambs

	Diet[1]			
Item	0% DDGS	20% DDGS	40% DDGS	60% DDGS
Ingredient, %	DM basis			
Alfalfa Hay	15.00	15.00	15.00	15.00
Corn	81.38	61.38	41.38	21.38
DDGS[2]	0.00	20.00	40.00	60.00
Ammonium Chloride	0.5	0.5	0.5	0.5
Limestone	2.25	2.25	2.25	2.25
Lasalocid[3]	0.085	0.085	0.085	0.085
TM package[4]	0.78	0.78	0.78	0.78
Copper Sulfate	0.002	0.002	0.002	0.002
Thiamin	0.011	0.011	0.011	0.011
Nutrient composition (analyzed)				
CP, %	14.0	19.4	22.0	24.7
NDF, %	23.7	27.6	30.6	31.8
ADF, %	10.1	11.0	11.1	11.5
S, %	0.22	0.52	0.70	0.84
Ca, %	1.72	1.64	1.35	1.16
P, %	0.50	0.65	0.77	0.81
Cu, mg/kg	19	19	15	17
Zn, mg/kg	59	95	90	73
Thiamin[5], mg/kg	70.8	67.2	55.5	51.5

[1] Diets were balanced to meet or exceed requirements set by (NRC, 2007). Treatments based on distillers dried grains with solubles inclusion: 1) 0% DDGS, 2) 20% DDGS), 3) 40% DDGS, 4) 60%DDGS.
[2] Distillers dried grains with solubles.
[3] Lasalocid (Bovatec 68, Alpharma Inc., Fort Lee, NJ).
[4] Trace Mineral (TM) package contained: 11.7% Ca, 10.0% P, 14% salt, 0.1% K, 0.1% Mg, 20 mg/kg Co, 100 mg/kg I, 2,450 mg/kg Mn, 50 mg/kg Se, 2,700 mg/kg Zn, 300,000 IU/lb Vitamin A, 30,000 IU/lb Vitamin D_3, and 600 IU/lb Vitamin E.
[5] Formulated based on estimated feed intake of 1.36 $kg \cdot hd^{-1} \cdot d^{-1}$, amount of supplemental thiamin provided, and corrected for thiamin contained in remaining feed ingredients.

Procedures for ruminal gas cap sampling were adapted from those of Gould et al. (1997). In order to obtain ruminal gas cap samples, wool was shorn from a 15 cm by 15 cm area of the animals left side immediately posterior to the 13th rib. Shearing was done with surgical clippers with care taken to remove all wool.

Table 3.3. Adaptation diets (%, DM basis) fed to lambs on d 0 - 28

	Diet					
	Arrival	Step 1	Step 2	Step 3	Step 4	Step 5
	d					
Ingredient	-6	0	7	14	21	28
0% DDGS						
Alfalfa Hay	46	46	46	35	25	15.0
Corn	50.4	50.4	50.4	61.4	71.4	81.4
DDGS[1]	0	0	0	0	0	0
Supplement[2]	3.6	3.6	3.6	3.6	3.6	3.6
20% DDGS						
Alfalfa Hay	46	46	46	35	25	15
Corn	50.4	50.4	45.4	51.4	56.4	61.4
DDGS[1]	0	0	5	10	15	20
Supplement[2]	3.6	3.6	3.6	3.6	3.6	3.6
40% DDGS						
Alfalfa Hay	46	46	46	35	25	15
Corn	50.4	50.4	40.4	41.4	41.4	41.4
DDGS[1]	0	0	10	20	30	40
Supplement[2]	3.6	3.6	3.6	3.6	3.6	3.6
60% DDGS						
Alfalfa Hay	46	46	46	35	25	15
Corn	50.4	50.4	35.4	31.4	26.4	21.4
DDGS[1]	0	0	15	30	45	60
Supplement[2]	3.6	3.6	3.6	3.6	3.6	3.6

[1] Distillers dried grains with solubles.
[2] Supplement contained (% total diet): 0.5% ammonium chloride, 2.25% limestone, 0.085% Lasalocid (Bovatec 68), 0.78% trace mineral, 0.002% copper sulfate, and 150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin.

After shearing, this area was scrubbed and disinfected with alternating isopropyl alcohol and Betadine scrubs. In order to accomplish multiple samples while maintaining the integrity of the rumen gas, two separate portions of the sampling apparatus were developed (Neville et al., 2010a). The first portion included the 7.6 cm 12 gauge needle which was connected to a 20 cm (4.75 mm diam.) tubing (Tygon ®, S-50-HL Class VI) via a Luer-lock connection. The second portion of the sampling apparatus included a 140 mL catheter tip syringe (Monoject, Sherwood Medical, Ballymoney, N. Ireland) which was connected

to an 8 cm (4.75 mm diam.) portion of tubing via Luer-lock connection. The two portions were then connected or disconnected through Luer-lock connections with ratchet tubing clamps utilized on both sides of the Luer-lock connectors. After the needle was introduced thru the skin and into the rumen gas cap a 120 mL sample (approximately) of ruminal gas was drawn into the syringe. The first of two syringes was then disconnected and a second filled in the same manner. Hydrogen sulfide gas detector tubes (Gastec©, Kanawaga Japan) were connected to a volumetric gas sampling pump and a volume (100 mL) was drawn through the detector tube to acquire a measurement of ruminal gas cap hydrogen sulfide. At each sampling point duplicate measurements were taken from each lamb and the average of the two samples was used for any calculations. If the detector tube failed to reach 100 ppm H_2S (the lowest detectable concentration recommended by the manufacturer) the reading was recorded as a zero. Following gas and fluid sampling, the needle was removed and the sampling site was sprayed with a 10% Iodine solution. Lambs were then given injections of penicillin (3 ml/d; Pro-Pen-G, Bimeda Inc., LeSueur, MN) for 3 consecutive d following sampling to prevent peritonitis. Ruminal H_2S concentrations were converted from parts per million to grams per cubic meter hydrogen sulfide through the following equation: H_2S (g/m^3) = [(H_2S (ppm) × 139.06)/1000000] assuming standard temperature and pressure values (Neville et al., 2010a).

Sulfur Balance.

On d 35, lambs were placed into metabolism crates and adapted to the crates for 10 d. Following adaptation, lambs were fitted with fecal collection bags. Collection of all samples as well as feed and water samples were conducted daily at 0700. Feed intake was recorded daily, with daily adjustments made to target ad libitum intake (10% feed

remaining). Ort samples were collected, weighed, and dried before being composited on an equal weight basis (10g/d) within lamb for laboratory analysis. Water was filled twice daily in order to provided ad libitum access. Water intake was calculated by subtracting any unconsumed water measured (volume) from water offered. Daily water samples were collected and frozen (-20°C) before being composited for laboratory analysis of water sulfates. Water was analyzed by a commercial laboratory (Stearns DHIA, Sauk Centre, MN) for sulfate (93 mg/L). Fecal bags were emptied daily, total feces weighed and 10% (wet weight) added to a composite sample which was frozen (-20°C) for later analysis. Plastic buckets (3.78 L) were placed beneath false-bottom metabolism crates to facilitate collection of urine. Urine buckets were acidified with 150 mL hydrochloric acid (50% w/v) to inhibit microbial growth and prevent volatilization. Urine output was filtered through 4 layers of cheesecloth before volume (mL) and weight (g) were recorded; a 10% subsample of urine weight was composited and frozen for later analysis.

Laboratory Analysis

Feed and ort samples were dried using a forced-air oven (55°C; The Grieve Corporation, Round Lake, IL) for 48 h. Dried samples were ground using a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 2 mm screen. Feed samples were analyzed for DM, ash, N, P; and Ca, Cu, and Zn (methods 934.01, 942.05, 2001.11, 965.17; and 968.08 respectively; AOAC, 2010). Concentrations of NDF (Van Soest et al., 1991; as modified by Ankom Technology, Fairport, NY) and ADF (Goering and Van Soest, 1970, as modified by Ankom Technology) were determined using an Ankom 200 Fiber Analyzer (Ankom Technology) without sodium sulfite, with amylase, and without ash corrections as sequentials. Sulfur and thiamin were analyzed by inductively coupled argon plasma and

AOAC procedure 942.23/HPLC, respectively, by a commercial laboratory (Midwest Laboratories, Omaha, NE).

Statistical Analysis

Hydrogen sulfide gas and pH data were analyzed utilizing the repeated measures analysis in the Mixed Procedures of SAS (SAS Inst. Inc., Cary, NY) with P-values ≤ 0.05 considered significant. Treatment, day, and the treatment by day interaction were evaluated. The covariate structure used was autoregressive [AR(1)]. Other structures were tested; however autoregressive was the best fit. Sulfur balance data were analyzed as a completely randomized design using the Mixed procedures of SAS with lamb serving as the experimental unit. The model included treatment. Linear and quadratic contrasts were used to evaluate the effect of increasing concentration of DDGS inclusion. Significance was declared at $P \leq 0.05$. In the case that a significant F-test was not observed but a contrast P-value was significant the difference will be discussed as a tendency.

Results and Discussion

Ruminal pH and Hydrogen Sulfide Concentration

Hydrogen sulfide gas concentration was affected by treatment, day, and a day by treatment interaction ($P < 0.001$; Figure 3.1). Hydrogen sulfide gas concentrations did not differ ($P \geq 0.06$) until d 14 when lambs fed 60% DDGS had greater H_2S concentrations (0.23 ± 0.039 g/m^3; $P \leq 0.006$) than all other treatments. Lambs fed 60% DDGS continued to have greater ($P \leq 0.001$) H_2S gas concentrations throughout the adaptation phase compared to the other treatments. Peak H_2S concentrations (0.01, 0.08, and 0.49 g/m^3) occurred on d 14, 21, and 28, respectively, for the 0 20, and 60% DDGS treatments. Peak concentrations were not established for those lambs fed 40% DDGS as no clear drop in H_2S

concentration was detected. Ruminal pH (data not shown) was not affected by a day x treatment interaction ($P = 0.65$) or by treatment ($P = 0.32$), but decreased ($P < 0.001$) across the adaptation phase from 5.82 (d -7) to 5.33 (d 35).

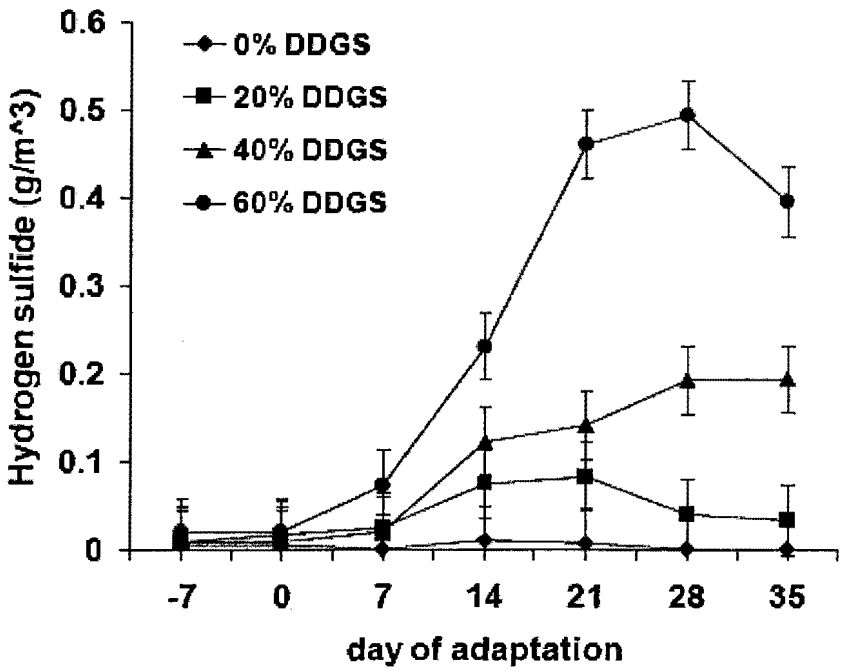

Figure 3.1. Influence of increasing concentrations (g/m^3) of distillers dried grains with solubles DDGS on ruminal hydrogen sulfide concentrations in lambs. *P*-values for effect of treatment ($P < 0.001$), day ($P < 0.001$), and treatment by day interaction ($P < 0.001$). Treatment diets were based on increasing the concentration of DDGS (0, 20, 40, or 60% of dietary dry matter). Concentrations of hydrogen sulfide gas measured via rumenocentesis on hydrogen sulfide detector tubes (Gastec, Kanawaga, Japan).

While the above data shows a clear increase in ruminal H_2S gas concentration with increasing DDGS inclusion there is substantial variation between animals. Lambs fed 60% DDGS in the present study had ruminal H_2S concentrations nearly half of those reported by Neville et al. (2010a) in finishing lambs fed diets similar in dietary and nutrient composition. Analyses of water sulfate concentrations were 74 and 93 mg/L for Neville et

al. (2010a) and the present study, respectively, so it is unlikely differences in water sulfate contributed greatly to the differences between the studies. Dietary S concentrations for the 60% DDGS treatment in the two studies were 0.71 to 0.84% S for Neville et al. (2010a) and the present study, respectively. Given that feed and water S concentrations as well as feeding regimen and dietary adaptation were similar in these two studies the differences in H_2S concentrations between the two studies may be a result of differences in sulfate reducing bacteria population in the rumen. Another potential explanation could be differences in form of S in the diet. While we did not measure S in it's various forms (amino acids, sulfate, etc.) differences in S form may be occurring within the DDGS. Further, decreases in ruminal pH coincide with increasing ruminal hydrogen sulfide concentrations supporting previous research. Gould (1998) suggested that sulfide in rumen fluid, ruminal fluid pH, frequency of eructation, and absorption of sulfide through the rumen mucosa may explain differences in ruminal H_2S concentrations.

Sulfur Balance

In our study, level of dietary DDGS inclusion did not affect DMI (1.37 ± 0.07 $kg \cdot hd^{-1} \cdot d^{-1}$; $P = 0.25$; Table 3.4). Zinn et al. (1997) reported that increasing levels of ammonium sulfate affected DMI and ADG in feedlot cattle. Kandylis et al. (1984) also reported a number of studies in both beef cattle and lambs that demonstrated DMI was reduced when feeding 0.3 to 1.2% dietary S. Qi et al. (1993) reported that DMI of growing goats peaked when dietary S was 0.2%. The present study contradicts these findings in that increasing S from DDGS did not result in decreased intake when dietary S exceeded 0.22%. However, the source of S (calcium sulfate vs. DDGS) as well as the range of S concentration evaluated likely influenced these findings and explain in part differences

between the two studies. Another possible explanation for these discrepancies includes differences in dietary ingredients. Most importantly, Qi et al. (1993) included 1.5% urea-N which resulted in a N:S ratio of 10:1 which is the recommended ratio (NRC, 2007) for lambs. The reason for the 10:1 recomenndation is to ensure enough S is present to allow for microbial production of S-amino acids. In the present study, our N:S ratios were 10:1, 6:1, 5:1, and 4.7:1 for the 0, 20, 40, and 60% DDGS diets, respectively. Zinn et al. (1997) also indicated differences in ruminal and total tract availability of S may influence animal performance. The present study, along with results of Schauer et al. (2008), appear to indicate that diets which include up to 60% DDGS (%, DM basis) do not result in reduced DMI or growth performance in growing and finishing lambs. Decreases in ruminal and intestinal motility (Bird, 1972; Kandylis, 1984) could explain the decreased DMI observed with increasing DDGS inclusion in other studies. Loneragan et al. (2001) hypothesized that either decreased gut motility or hepatic injury may reduce animal performance. Liver function was not assessed in the present study; therefore it is possible that liver metabolism could have been impacted. Unpublished data from a concurrent project (Neville et al., 2010b) found no liver abscess in steers fed increasing concentrations of DDGS (dietary S levels > 0.6% S). However, presence of liver abscesses may be dependent on rate of dietary adaptation and use of antimicrobial compounds, such as Tylosin (Nagaraja and Chengappa, 1998; Vasconcelos and Galyean, 2008) and should not be viewed entirely as indicators of liver function.

Sulfur intake from feed and water, as well as S excretion in feces and urine increased linearly ($P \leq 0.009$) with increasing DDGS in the diet. Lambs fed 60% DDGS had water intakes 54% greater than those fed no DDGS ($P < 0.01$). Increased water intake

resulted in an increase of 3 fold in urine volume and a 4.8 fold increase in urinary S excretion ($P < 0.01$) compared to lambs fed no DDGS. Given the water intake and urine output data, ad libitum access to low sulfate water may be key to preventing S toxicity when high amounts of DDGS are fed to growing and finishing lambs. Sulfur is primarily excreted as sulfate in the urine or as organic S in feces (Underwood and Suttle, 1999). Net S balance increased linearly ($P = 0.004$) with increasing inclusion of DDGS in finishing diets. Actual S balance is not reported as the total volume of eructated H_2S gas was not measured. It is likely that substantial amounts of S were also excreted via rumen gases through eructation. Therefore, further research is needed to quantify S excretion via H_2S gas and eructation. The present study serves as another example of the need to quantify H_2S lost via eructation, and more importantly, quantify H_2S inhalation after eructation.

The scientific literature is lacking in defining the various forms of S contained within DDGS. Quantifying proportions of the various forms of S will undoubtedly add to the current literature and assist in determination of mechanisms of S-toxicity in the ruminant animal. Additionally, determining how digestibility or availability of S in its various forms influences S reduction and creation of hydrogen sulfide gas within the rumen will further aid in the understanding of S-toxicity mechanisms.

Kandylis (1984) reported that H_2S present in the rumen may cause neurological or respiratory distress. As in our previous research (Neville et al., 2010a), we did not observe any outward clinical signs of PEM. There is great disparity in the literature regarding differences between concentration and total production of ruminal H_2S. The present study reports H_2S in terms of concentration. The values for S retention give some indication of the quantity of hydrogen sulfide excreted by the animal. This data does not account for the

use of S in production of wool, muscle tissue, or other protein (S-amino acid) production which would also impact calculations of S retention.

Increasing concentration of DDGS in the diet increased S intake, excretion, and H_2S gas concentrations but did not result in the occurrence of PEM. Understanding that S excretion increased with increasing dietary S concentrations explains, in part, why S toxicity did not occur. Continued efforts to quantify H_2S production will add to the body of knowledge regarding S metabolism and excretion. The present study, along with previous research at our institution, has demonstrated that feeding up to 60% dietary DDGS concentrations is possible without affecting lamb health or performance. Clearly, defining S source as a determining factor of S toxicity is needed in the scientific literature. Accounting for digestibility or availability of various S sources will facilitate in more appropriately defining both maximum tolerable and toxic levels in future recommendations.

Table 3.4. Intake, excretion, and sulfur balance of lambs fed increasing concentrations of distillers dried grains with solubles

	Treatment[1]						*P*-Value[3]	
Item	0% DDGS	20% DDGS	40% DDGS	60% DDGS	SEM[2]	*P*-value	Linear	Quadratic
Intake								
Feed, kg DM	1.3	1.5	1.4	1.3	0.07	0.25	0.68	0.06
S, mg	2,487.5	6,076.2	7,429.4	9,029.6	816.6	<0.001	<0.001	0.25
Water, L	3.1	3.5	3.7	4.8	0.28	0.006	<0.001	0.31
S, mg	94.8	109.4	115.7	148.9	8.7	0.006	0.001	0.31
Total S, mg	2,582.4	6,185.6	7,545.1	9,178.4	815.8	<0.001	<0.001	0.25
Excretion								
Fecal, kg DM	0.20	0.23	0.27	0.25	0.02	0.17	0.06	0.33
S, mg	761.4	947.6	1112.1	1130.5	90.6	0.05	0.009	0.37
Urine, L	0.59	0.85	1.1	2.4	0.3	0.008	0.002	0.12
S, mg	674.9	2,370.8	3,236.0	3,945.1	268.8	<0.001	<0.001	0.09
Total S, mg	1,436.3	3,318.4	4,348.0	5,075.6	344.5	<0.001	<0.001	0.12
Sulfur Balance, mg	1,146.1	2,867.2	3,197.1	4,102.8	568.0	0.02	0.004	0.49

[1] DDGS = Distillers dried grains with solubles.
[2] n = 4.
[3] *P*-value for linear and quadratic effects of increasing concentration of DDGS in diet.

Literature Cited

AOAC. 2010. Official Methods of Analysis. 18th ed. Assoc. of Offic. Anal. Chem. Arlington, VA.

Bird, P. R. 1972. Sulphur metabolism and excretion studies in ruminants X. Sulphide toxicity in sheep. Aust. J. Biol. Sci. 25:1073-1085.

Dougherty, R. W., C. H. Mullenax, and M. J. Allison. 1965. Physiological phenomena associated with eructation in ruminants. In: Physiology of digestion in the ruminant. Butterworths. Washington, D.C.

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.

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

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

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

Lamm, C. G., G. R. Richter, J. D. Taylor, and S. E. Morgan. 2010. Characterization of sulfur and sulfate toxicosis in cattle. Bov. Pract. 44(2):124-130.

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.

Low, J. C., P. R. Scott, F. Howie, M. Lewis, J. FitzSimons, and J. A. Spence. 1996. Sulphur-induced polioencephalomalacia in lambs. Vet. Rec. 138:327-329.

Nagaraja T. G., and M. M. Chengappa. 1998. Liver abscesses in feedlot cattle: A review. J. Anim. Sci. 76:287-298.

National Research Council. 2005. Mineral tolerances of animals, Second revised edition. The National Academies Press. Washington, D.C.

National Research Council. 2007. Nutrient requirements of small ruminants. The National Academies Press. Washington, D.C.

Neville, B. W., C. S. Schauer, K. Karges, M. L. Gibson, M. M. Thompson, L. A. Kirschten, N. W. Dyer, P. T. Berg, and G. P. Lardy. 2010a. Effect of thiamine supplementation on animal health, feedlot performance, carcass characteristics, and ruminal hydrogen sulfide concentrations in lambs fed diets based on 60% distillers dried grains plus solubles. J. Anim. Sci. 88:2444-2455.

Neville, B. W., C. S. Schauer, K. Karges, S. R. Eckerman, P. T. Berg, and G. P. Lardy. 2010b. Impact of corn processing on health and performance of steers fed increasing concentrations of distillers dried grains plus solubles. (Unpublished data)

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.

Schauer, C. S., M. M. Stamm, T. D. Maddock, and P. B. Berg. 2008. Feeding 60% of lamb finishing rations as dried distillers grains with solubles results in acceptable performance and carcass quality. Sheep and Goat Res. J. 23:15-19.

Underwood, E. J., and N. F. Suttle. 1999. The mineral nutrition of livestock. 3rd Ed. CABI Publishing. Cambridge, MA.

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

Vasconcelos, J. T., and M. L. Galyean. 2008. ASAS Centennial Paper: Contributions in the Journal of Animal Science to understanding cattle metabolic and digestive disorders. J. Anim. Sci. 86:1711-1721.

Zinn, R. A., E. Alvarez, M. Mendez, M. Montaño, 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.

Zinn, R. A., E. Alvarez, M. Montaño, and E. Ramirez. 1999. Toxic effects of high dietary sulfur on growth performance of feedlot calves during the early growing phase. Proc. W. Sec. Am. Soc. Anim. Sci. 50:356-358.

CHAPTER IV. IMPACT OF CORN PROCESSING ON HEALTH AND PERFORMANCE OF STEERS FED INCREASING CONCENTRATIONS OF DISTILLERS DRIED GRAINS PLUS SOLUBLES

Abstract

Feeding increased concentrations of distillers dried grains plus solubles (**DDGS**) to ruminants has been avoided due to risks of sulfur toxicity and concerns about animal performance. The objective of this study was to evaluate the influence of feeding increasing concentration of DDGS and corn processing method on animal performance, incidence of polioencephalomalacia, and concentration of H_2S gas in feedlot steers. Seventy-two steer calves (340 ± 12.2 kg) were individually fed for an average of 105 d and utilized in a completely random design with a 3 x 2 factorial arrangement of treatments. Main effects included concentration of DDGS (20, 40, or 60% DM basis) and corn processing method [high-moisture (**HMC**) vs. dry-rolled corn (**DRC**)] resulting in treatments of: 1) 20% DDGS with DRC, 2) 40% DDGS with DRC, 3) 60% DDGS with DRC, 4) 20% DDGS with HMC, 5) 40% DDGS with HMC, and 6) 60% DDGS with HMC. Ruminal H_2S gas concentrations were measured on d 0, 7, 14, 21, 28, 35, 49, 63, and 91 via rumen puncture. Animal performance and carcass characteristic data were collected. The day × corn processing × DDGS interaction for H_2S gas concentrations was not significant ($P = 0.91$). Ruminal H_2S concentration was affected by increasing DDGS concentration ($P < 0.001$) and day ($P < 0.001$), but not by corn processing method ($P = 0.94$). During the finishing phase, ADG and DMI decreased quadratically ($P \leq 0.02$) while G:F decreased linearly ($P = 0.01$) with increasing concentration of DDGS. Final BW decreased linearly ($P = 0.002$) with increasing DDGS inclusion. Carcass composition reflected the decrease in final BW with decreased HCW ($P = 0.006$), as well as decreased

fat depth ($P = 0.005$) with increasing concentrations of DDGS. The combination of decreased HWC and backfat thickness resulted in decreased ($P = 0.01$) yield grade with increasing DDGS inclusion. There were no confirmed cases of polioencephalomalacia. In conclusion, corn processing did not influence animal performance, incidence of polioencephalomalacia, or H_2S concentrations under the conditions of this study. Feeding 60% DDGS in beef cattle finishing diets is not recommended due to poor animal performance.

Introduction

One challenge with using ethanol co-products is the potential for sulfur induced polioencephalomalacia (**PEM**) in ruminants (Gould, 1998). Research has demonstrated that lambs fed diets containing 60% distillers dried grains with solubles (**DDGS**) did not develop PEM (Neville et al., 2010) and performed similar to those fed lesser concentrations of DDGS (Schauer et al., 2008). Schauer et al. (2008) and Neville et al. (2010) provide evidence that use of DDGS can be increased in lamb finishing rations. Average utilization of DDGS in the beef feedlot industry is 16.5% (Vasconcelos and Galyean, 2007); this is lower than the 20 to 30% inclusion suggested to optimize ADG and G:F (Klopfenstein et al., 2008). The reason for the low DDGS inclusion rate in beef feedlot diets could be economic or more likely a result of negative connotations with feeding co-products (i.e. sulfur content). Feeding 60% DDGS (average S content of DDGS 0.6 to 1.0%; Klopfenstein et al., 2008) will result in exceeding the maximum tolerable level of dietary S (0.3%; NRC, 2005).

Neville et al. (2010) demonstrated that lambs can be fed a greater S concentration than recommended by NRC (2005). We hypothesized feeding combinations of DDGS and

either dry-rolled corn (**DRC**) or high-moisture corn (**HMC**) with dietary S content exceeding 0.3% S will not result in incidence of PEM. We further hypothesized that feeding HMC in combination with DDGS will increase ruminal hydrogen sulfide (H_2S) gas concentrations over those found when feeding DDGS with DRC. Admittedly animal performance may suffer due to decreased palatability and intake as the concentration of DDGS increases. However in some situations, the economic benefit from decreased feed costs may warrant such feeding practices. The objective of this study was to evaluate the influence of feeding increasing concentrations of DDGS and corn processing method (HMC vs. DRC) on animal performance, incidence of PEM, and concentration of ruminal H_2S in feedlot steers.

Materials and Methods

All animal care and handling procedures were approved by the North Dakota State University Animal Care and Use Committee prior to the initiation of the research.

Animals and Treatments

Seventy-two mixed breed steer calves (340 ± 12.2 kg) were utilized in a completely random design with a 3 x 2 factorial arrangement of treatments to evaluate the outlined objective. Animals were assigned to treatment at the time of arrival. Main effects included concentration of DDGS (20, 40, or 60% DM basis) and corn processing method [high-moisture (HMC) vs. dry-rolled corn (DRC)] resulting in treatments of: 1) 20% DDGS with DRC, 2) 40% DDGS with DRC, 3) 60% DDGS with DRC, 4) 20% DDGS with HMC, 5) 40% DDGS with HMC, and 6) 60% DDGS with HMC. Treatment diets were formulated to meet or exceed dietary nutrient requirements for steers weighing 324 kg and gaining 1.45 kg daily (NRC, 2000; Table 4.1). The dietary treatments were formulated to have

minimum Ca to P ratio of 1:1. Diets were formulated to provide 150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamine based on an estimated DMI of 10 kg; actual thiamine content of diet samples was 13.55 mg/kg (135.5 $mg \cdot hd^{-1} \cdot d^{-1}$).

Table 4.1. Ingredient and nutritional composition of final finishing diets fed to steers

	Dry-Rolled Corn			High-moisture Corn		
Item	20% DDGS	40% DDGS	60% DDGS	20% DDGS	40% DDGS	60% DDGS
Ingredient, %						
Alfalfa Hay	5.0	5.0	5.0	5.0	5.0	5.0
Corn Silage	10.0	10.0	10.0	10.0	10.0	10.0
Corn[1]	58.2	38.2	18.2	58.2	38.2	18.2
DDGS[2]	20.0	40.0	60.0	20.0	40.0	60.0
CSB[3]	5.0	5.0	5.0	5.0	5.0	5.0
Supplement[4]	1.8	1.8	1.8	1.8	1.8	1.8
Nutrient composition, % (analyzed)						
CP	15.9	20.8	22.6	16.1	19.6	23.0
NDF	25.5	30.1	31.7	24.6	27.6	30.9
ADF	7.7	8.8	8.6	7.8	8.5	8.5
Ca	1.1	0.9	0.7	0.9	0.9	0.7
P	0.6	0.7	0.9	0.5	0.7	0.8
S	0.6	0.7	0.9	0.6	0.7	0.9
Cu	0.003	0.003	0.002	0.003	0.003	0.003
Zn	0.01	0.1	0.1	0.1	0.1	0.1

[1] Corn fed either as dry-rolled corn or high-moisture corn.
[2] DDGS = distillers dried grains plus solubles.
[3] CSB = concentrated separator byproduct.
[4] Supplement contained (%, total ration, DM basis): limestone 1.7%; vitamin A, D, and E premix 0.02% [Trouw Nutrition, Highland, IL (1,500,000 IU vitamin A, 500,000 IU vitamin D, and 500 IU vitamin E)]; Rumensin 0.02% (176 g/kg Monensin, Elanco Animal Health, Indianapolis, IN); Trace mineral premix 0.05% [Hubbard Feeds Inc., Mankato, MN (3.95% Ca, 2.56% Cu, 16.0% Zn, 4.0% Mn, 1,050 mg/kg I, and 250 mg/kg Co)]; 0.002% thiamin (analyzed concentration 13.55 mg/kg dietary DM).

Prior to initiation of this study steers were vaccinated for clostridial disease (Convexin 8; Schering-Plough, Kenilworth, NJ) at one d of age. Additionally, steers were vaccinated for respiratory disease (Bovi-Shield GOLD 5; Pfizer Animal Health, New York, NY), clostridial disease (One-Shot Ultra 7; Pfizer Animal Health, New York, NY) and dewormed (Dectomax Injectable, Pfizer Animal Health, New York, NY) at 4 mo of age. Steers were revaccinated at weaning (appox. 6 to 7 mo of age) for respiratory disease (Bovi-Sheild GOLD 5), clostridial disease (Ultrabac 7 with Somubac; Pfizer Animal

Health, New York, NY) and dewormed (Ivomec Pour-On; Merial, Duluth, GA). Steers were maintained for 56 d on a medium concentrate hay/corn silage-based diet until initiation of study procedures. Steers were trained to use the Calan Broadbent Feeding System (American Calan, Northwood, NH) prior to adaptation to finishing diets. During this training phase steers were fed a diet consisting of 50% corn silage, 25% alfalfa hay, 25% dry-rolled corn (DM basis). Steers were maintained on this diet until d 0 at which time adaptation to final finishing diets began. Neither the receiving diet nor the training diet contained DDGS. Calan facilities are located in a temperature controlled building maintained at 15° C, daylight conditions were simulated with 12 h of lighting. Pens had slatted concrete floors and are 5.0 by 5.3 m with 6 steers housed in each pen

Ruminal Hydrogen Sulfide Gas Sampling

Ruminal H_2S gas concentrations were measured via rumen puncture during the adaptation to the finishing diets and throughout the finishing phase on 4 steers from each treatment. Collection of rumen gasses occurred 5 h after feed was offered. Hydrogen sulfide measurements were collected on d 0, 7, 14, 21, 28, 35, 49, 63, and 91; final finishing diets were provided on d 28. On d 0, steers began the dietary adaptation period which increased the concentrate portion of the diet to 85% over 28 d (Table 4.2). Adaptation diets increased the amount of concentrate (corn and DDGS) while reducing the amount of corn silage and alfalfa hay.

Procedures for ruminal gas cap sampling were adapted from those of Gould et al. (1997). In order to obtain ruminal gas cap samples, hair was clipped from a 15 cm by 15 cm area of the animals left side immediately posterior to the 13^{th} rib. Clipping was done with surgical clippers with care taken to remove all hair. After clipping, this area was

scrubbed and disinfected with alternating isopropyl alcohol and Betadine scrubs. In order to obtain multiple samples while maintaining the integrity of the rumen gas, two separate portions of the sampling apparatus were developed (Neville et al., 2010). The first portion included the 10.2 cm 14 gauge needle which was connected to a 20 cm (4.75 mm diam.) tubing (Tygon ®, S-50-HL Class VI) via a Luer-lock connection.

Table 4.2. Final finishing ration and adaptation diets (%, DM basis) fed to steers

	Stage of Adaptation				
Diet	Step 1	Step 2	Step 3	Step 4	Step 5
Day	0	7	14	21	28
20% DDGS					
Alfalfa Hay	20.0	16.3	12.5	8.8	5.0
Corn Silage	40.0	32.5	25.0	17.5	10.0
DDGS[1]	--	5.0	10.0	15.0	20.0
Corn[2]	33.2	39.4	45.7	51.9	58.2
CSB[3]	5.0	5.0	5.0	5.0	5.0
Supplement[4]	1.8	1.8	1.8	1.8	1.8
40% DDGS					
Alfalfa Hay	20.0	16.3	12.5	8.8	5.0
Corn Silage	40.0	32.5	25.0	17.5	10.0
DDGS[1]	--	10.0	20.0	30.0	40.0
Corn[2]	33.2	34.4	35.7	36.9	38.2
CSB[3]	5.0	5.0	5.0	5.0	5.0
Supplement[4]	1.8	1.8	1.8	1.8	1.8
60% 0DDGS					
Alfalfa Hay	20.0	16.3	12.5	8.8	5.0
Corn Silage	40.0	32.5	25.0	17.5	10.0
DDGS[1]	--	15.0	30.0	45.0	60.0
Corn[2]	33.2	29.4	25.7	21.9	18.2
CSB[3]	5.0	5.0	5.0	5.0	5.0
Supplement[4]	1.8	1.8	1.8	1.8	1.8

[1] DDGS = distillers dried grains plus solubles.
[2] Corn fed either as dry-rolled corn or high-moisture corn.
[3] CSB = concentrated separator byproduct.
[4] Supplement contained (%, total ration DM basis): limestone 1.7%; vitamin A, D, and E premix 0.02% [Trouw Nutrition, Highland, IL (1,500,000 IU vitamin A, 500,000 IU vitamin D, and 500 IU vitamin E)]; Rumensin 0.02% (176 g/kg Monensin, Elanco Animal Health, Indianapolis, IN); Trace mineral premix 0.05% [Hubbard Feeds Inc., Mankato, MN (3.95% Ca, 2.56% Cu, 16.0% Zn, 4.0% Mn, 1,050 mg/kg I, and 250 mg/kg Co)]; 0.002% thiamin (analyzed concentration 13.55 mg/kg dietary DM).

The second portion of the sampling apparatus included a 140 mL catheter tip syringe (Monoject, Sherwood Medical, Ballymoney, N. Ireland) which was connected to an 8 cm (4.75 mm diam.) portion of tubing via Luer-lock connection. The two portions were then connected or disconnected through Luer-lock connections with ratchet tubing clamps utilized on both sides of the Luer-lock connectors. After the needle was introduced through the skin and into the rumen gas cap a 120 mL sample (approximately) of ruminal gas was drawn into the syringe. The first of two syringes was then disconnected and a second filled in the same manner. Hydrogen sulfide gas detector tubes (Gastec©, Kanawaga Japan) were connected to a volumetric gas sampling pump and a volume (100 mL) was drawn through the detector tube to acquire a measurement of ruminal gas cap H_2S. At each sampling point, duplicate measurements were taken from each steer and the average of the two samples was used for any calculations. If the detector tube failed to reach 100 ppm hydrogen sulfide (the lowest detectable concentration recommended by the manufacturer) the reading was reported as a zero. Following gas sampling, the needle was removed and the sampling site was sprayed with a 10% iodine solution. Steers were then given a single 10 mL injection of penicillin (Pro-Pen-G, Bimeda Inc., LeSueur, MN) to prevent infection after conclusion of gas sampling procedure. Ruminal H_2S concentrations were converted from parts per million to grams per cubic meter H_2S through the following equation: H_2S $(g/m^3) = [(H_2S\ (ppm) \times 139.06)/1000000]$ assuming standard temperature and pressure values (Neville et al., 2010).

Feeding Study

Two day body weights were collected at arrival (d -28), beginning of dietary adaptation (d 0), beginning of the finishing phase (d 28), and the conclusion of the study

(approx. d 105). Interim weights were collected every 28 d as single day weights to monitor animal performance (data not presented). Steers received a single implant containing 80 mg trenbolone acetate and 16 mg estradiol (Revalor-IS, Intervet Inc., Millsboro, DE) on d 28. Feed offered was recorded daily with feed refusals collected, weighed, and sampled weekly. Weekly feed samples were collected to determine dietary DM and nutrient composition. Average daily gain and G:F were calculated based on these data.

Backfat thickness was measured via ultrasound to determine market readiness of steers. Steers were sent to either the NDSU Meats Laboratory or a commercial abattoir (Tyson Fresh Meats Inc., Dakota City, NE) for harvest. Eight steers were harvested at the NDSU Meats Laboratory in groups of 4 on d 64 and 99. This constituted 2 animals from the HMC with 20% DDGS and DRC with 40% DDGS treatments and 1 animal each from the remaining 4 treatments. The remaining animals were shipped on d 127 or 156 based on ultrasonic estimates of backfat thickness. Hot carcass weights were collected within 30 min of exsanguination. Ribeye area and 12th-rib fat were measured directly while maturity, marbling score, and KPH were assessed visually and recorded by trained personnel. Liver scores were recorded with evaluation based on procedures outlined by Brink et al. (1990).

Laboratory Analysis

Feed and ort samples were dried using a forced-air oven (55°C; The Grieve Corporation, Round Lake, IL) for 48 h. Feed samples were analyzed for DM, ash, N, P; and Ca, Cu, and Zn (methods 934.01, 942.05, 2001.11, 965.17; and 968.08 respectively; AOAC, 2010). Concentrations of NDF (Van Soest et al., 1991; as modified by Ankom

Technology, Fairport, NY) and ADF (Goering and Van Soest, 1970, as modified by Ankom Technology) were determined using an Ankom 200 Fiber Analyzer (Ankom Technology) without sodium sulfite, with amylase, and without ash corrections as sequentials. Sulfur and thiamin were analyzed by inductively coupled argon plasma and AOAC procedure 942.23/HPLC, respectively, by a commercial laboratory (Midwest Laboratories, Omaha, NE).

Statistical Analysis

Hydrogen sulfide gas data were analyzed utilizing the repeated measures analysis in the Mixed Procedures of SAS (SAS Inst. Inc., Cary, NC) with P-values ≤ 0.05 considered significant. Corn processing, DDGS concentration, day, and all interactions were evaluated. The covariate structure used was Simple. Other structures were tested; however Simple was the best fit. Performance data were analyzed as a completely randomized design with a 3 x 2 factorial arrangement of treatments using the Mixed procedures of SAS with steer serving as the experimental unit. The model included effects of concentration of DDGS, corn processing, and the interaction of DDGS and corn processing. Linear and quadratic contrasts were used to evaluate the effect of increasing concentration of DDGS inclusion. Sixty-eight steers completed the study. The reasons for removal from study included: failure to adapt to calan gate feeding system (3 steers) and euthanasia due to joint deterioration (1 steer).

Results

Ruminal Hydrogen Sulfide

The day × corn processing × DDGS concentration interaction for hydrogen sulfide gas concentrations was not significant ($P = 0.91$). Ruminal H_2S concentration was affected

by increasing DDGS concentration in the diet ($P < 0.001$) and day ($P < 0.001$), but not by corn processing method ($P = 0.94$). No differences in H_2S concentration among treatments were observed on d 0, 7, 14, or 21 ($P \geq 0.14$; Figure 4.1). On d 28, steers fed 60% DDGS had greater ($P \leq 0.006$) H_2S concentrations than those fed either 20 or 40% DDGS. Hydrogen sulfide concentration increased ($P < 0.001$) from d 28 to d 91 for steers fed 60% DDGS. Steers fed 60% DDGS had the greatest concentrations of H_2S on d 91 (1.38 g hydrogen sulfide/m^3; $P \leq 0.01$). Hydrogen sulfide concentrations were either static ($P = 0.68$) or tended to decrease ($P = 0.08$) for steers fed 20 or 40% DDGS, respectively, from d 49 to d 91.

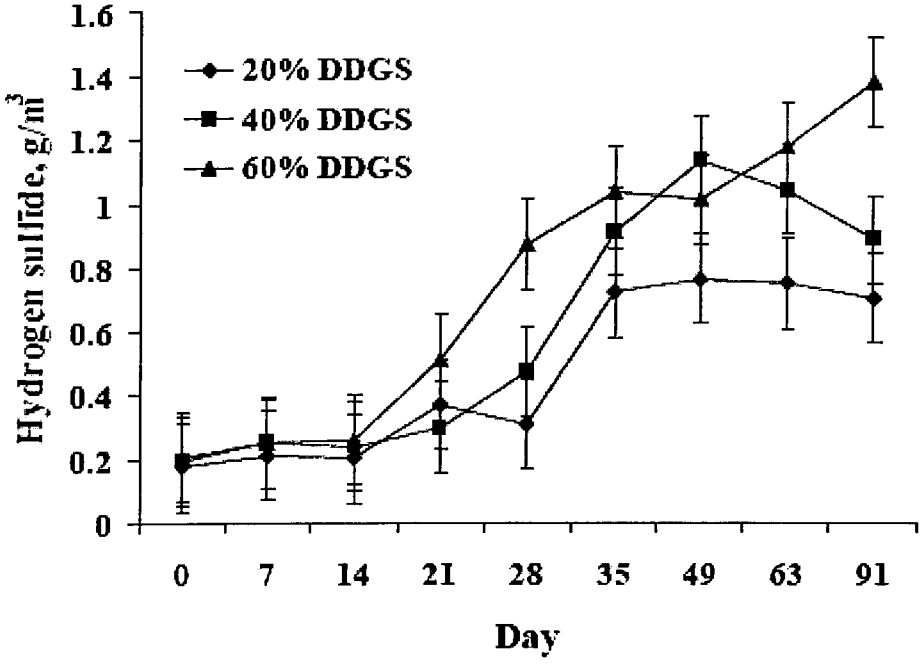

Figure 4.1. Change in hydrogen sulfide concentration (g/m^3) caused by increasing dietary distillers dried grains with solubles (DDGS) concentration in steers over adaptation from a medium-concentrate to high-concentrate finishing ration. Treatments were based concentrations of DDGS (20, 40, and 60% DM basis) as well as corn processing (high-moisture vs. dry-rolled corn). *P*-values: corn processing ($P = 0.94$), DDGS ($P < 0.001$), and corn processing by DDGS ($P = 0.36$).

Animal Performance

Results for steer performance are reported in Table 4.3. There were no corn processing and DDGS concentration interactions ($P \geq 0.12$). Furthermore, there was no effect of corn processing ($P \geq 0.14$). Therefore the effects will be discussed as either linear or quadratic responses to increasing DDGS concentration.

There were no differences in initial BW ($P \geq 0.82$) due to DDGS inclusion with steers averaging 340 ± 12 kg. Performance data was partitioned into adaptation (d 0 - 28) and finishing (d 29 - slaughter). During the adaptation phase there were no differences in ADG, DMI, or G:F for DDGS concentration ($P \geq 0.35$). During the finishing phase ADG and DMI decreased quadratically ($P \leq 0.02$) while G:F decreased linearly ($P = 0.01$) with increasing concentration of DDGS in the diet. As a result of decreased ADG, final BW decreased linearly ($P = 0.002$) with increasing DDGS inclusion.

Similar to steer performance, corn processing and DDGS concentration interactions were not affected ($P \geq 0.12$) and corn processing had no affect ($P \geq 0.35$) on carcass characteristics of steers (Table 4.4). Carcass composition reflected the decrease in final BW with a linear decrease in HCW ($P = 0.006$) as well as a linear decrease in fat depth ($P = 0.005$) with increasing concentration of DDGS in the diet. As a result of decreased backfat thickness, yield grade decreased linearly ($P = 0.01$) with increasing DDGS inclusion. Marbling score was unaffected by corn processing ($P = 0.46$) and DDGS concentration ($P = 0.82$) with an average marbling score of 477 ± 33.6 (Small^0 = 400). Further, KPH, ribeye area, and quality grade were unaffected by corn processing ($P \geq 0.35$) and DDGS concentration ($P \geq 0.18$). Liver abscess evaluation resulted in all scores of 0 (no abscesses).

Table 4.3. Influence of corn processing and concentration of distillers dried grains plus solubles (DDGS) on animal performance of steers

	Dry-Rolled Corn			High-moisture Corn				*P*-value [1,2]			
Item	20% DDGS	40% DDGS	60% DDGS	20% DDGS	40% DDGS	60% DDGS	SEM[3]	Corn	DDGS	L	Q
Initial BW, kg	345	340	345	340	341	340	13.0	0.76	0.97	0.96	0.82
Final BW, kg	622	606	589	616	621	559	14.1	0.52	0.004	0.002	0.16
Adaptation[4], d 0 - 28											
ADG, kg	1.8	1.8	1.7	1.7	1.8	1.8	0.10	0.87	0.64	0.91	0.35
DMI, kg	11.1	11.8	11.6	11.4	11.5	11.6	0.38	0.97	0.41	0.29	0.42
G:F	0.16	0.16	0.15	0.14	0.16	0.16	0.01	0.70	0.77	0.74	0.51
Finishing[5], d 29- Slaughter											
ADG, kg	2.0	1.9	1.4	2.0	1.8	1.2	0.10	0.14	<0.001	<0.001	0.02
DMI, kg	11.0	10.5	8.5	10.8	10.2	7.8	0.40	0.23	<0.001	<0.001	0.01
G:F	0.18	0.18	0.17	0.18	0.17	0.15	0.01	0.35	0.03	0.01	0.36

[1] *P*-values for effect of corn processing, concentration of DDGS, and linear or quadratic effect of DDGS.
[2] DDGS x corn processing interaction ($P \geq 0.17$); thus main effects of corn processing and DDGS inclusion are presented.
[3] n = 11, 12, 10, 12, 11, and 12, respectively.
[4] Adaptation measured from day 0 through day 28.
[5] Finishing measured from day 29 through slaughter.

Table 4.4. Influence of corn processing and concentration of distillers dried grains plus solubles (DDGS) on carcass quality of steers

	Dry-Rolled Corn			High-moisture Corn				*P*-value[1,2]			
Item	20% DDGS	40% DDGS	60% DDGS	20% DDGS	40% DDGS	60% DDGS	SEM[3]	Corn	DDGS	L	Q
HCW, kg	384.3	374.8	364.9	379.0	383.6	348.5	9.30	0.56	0.01	0.006	0.18
Fat depth, cm	1.1	1.0	0.9	1.2	1.1	0.8	0.10	0.78	0.01	0.005	0.29
KPH, %	1.9	2.0	2.0	1.8	2.0	1.7	0.14	0.35	0.18	0.99	0.07
Ribeye area, cm^2	85.2	82.6	84.5	83.9	90.3	83.2	2.45	0.40	0.56	0.86	0.29
Marbling score[4]	466	488	505	478	489	436	33.6	0.46	0.82	0.95	0.53
Quality Grade[5]	10.1	9.9	10.7	10.3	10.5	10.1	0.45	0.87	0.87	0.61	0.89
Yield Grade	3.0	3.0	2.6	3.0	2.8	2.4	0.19	0.45	0.03	0.01	0.48

[1] *P*-values for effect of corn processing, concentration of DDGS, and linear or quadratic effect of DDGS.
[2] DDGS x corn processing interaction ($P \geq 0.12$); thus main effects of corn processing and DDGS inclusion are presented.
[3] n = 11, 12, 10, 12, 11, and 12, respectively.
[4] Marbling score based on 400 = Small^0.
[5] Quality Grade based on Low Choice (Ch^-) = 10, High Prime (Pr^+) = 15.

Discussion

Previous research (Leibovich et al., 2009) has reported that corn processing did not affect H_2S production. Leibovich et al. (2009) further reported that an increase in available starch does not increase hydrogen sulfide gas production. While previous work focused on a low range (0 to 0.3%) dietary S content, the present study indicates that the impacts of corn processing are minimal at a greater range (0.6 to 0.9%) of dietary S concentrations. The present study did not evaluate the changes in ruminal H_2S production throughout the day (i.e. time relative to feeding), but instead measured concentration at a common time after feeding. However, previous work (May et al., 2009) does indicate such changes do occur in ruminal fermentation within a 24 h period when various concentrations of DDGS and corn processing methods are utilized in ruminant diets.

Unlike previous reports (Gould et al., 1997) the present study indicates that H_2S concentrations do not decrease immediately after adaptation to high concentrate rations. One possible explanation for this is differences in acute versus chronic exposure to sulfur. Further, the results from the present study indicate that rumen microorganisms do not adapt in a way that decreases the concentration of H_2S in the rumen gasses. Other work (Niles et al., 2002; Neville et al., 2010) feeding various concentrations of DDGS agree with the present study in that increasing DDGS concentration in the diet results in increased H_2S concentration in the rumen gas cap. Lacking in the literature are direct comparisons in H_2S concentrations between different species such as sheep and cattle. Further factors influencing results may include the use of feed additives, such as monensin, which have been shown to stimulate H_2S production (Kung et al., 2000). However, Quinn et al. (2009) reported that ionophores and antibiotics do not affect in vitro H_2S production. Research

clearly defining differences in species as well as the use of various feed additives may improve understanding the results of the present study.

Corrigan et al. (2009) reported that corn processing (DRC, HMC, or SFC) resulted in changes in DMI, however DMI was not impacted by corn processing in the present study. Similar to Corrigan et al. (2009) the present study demonstrated a quadratic decrease in DMI with increasing inclusion of DDGS. The type of distillers grains with solubles (wet vs. dry) as well as the corn processing methods evaluated vary between Corrigan et al. (2009) and those used in the present study, which may explain these differences.

Decreases in ruminal and intestinal motility (Bird, 1972; Kandylis, 1984) could explain the decreased DMI observed with increasing DDGS inclusion. Loneragan et al. (2001) hypothesized that either decreased gut motility or hepatic injury may reduce animal performance. Liver function was not assessed in our study and while no abscesses were observed, it is possible that other aspects of liver metabolism which we did not measure could have been impacted. However, this may be dependent on management and use of feed additives (Nagaraja and Chengappa, 1998; Vasconcelos and Galyean, 2008).

It is unknown if the decreases in intake and performance in the present study are a function of decreased gut motility or sub-clinical PEM. However, sub-clinical disease can decrease feedlot performance (Morck et al., 1993). Loneragan et al. (2001) concluded that while H_2S levels may not be great enough to cause toxicity, H_2S levels may be great enough to decrease animal growth by increasing the energy demands for detoxification in the liver. The results of the present study indicate steer performance was impacted when greater concentrations of DDGS were fed resulting in greater H_2S concentrations.

The decrease in HCW, backfat thickness, as well as yield grade for the steers fed 60% DDGS are understandable given the decreased performance of those animals. Corrigan et al. (2009) reported a similar relationship between carcass characteristics and feedlot performance. Although Corrigan's data demonstrated improved performance with increasing wet distillers grains with solubles inclusion up to 40% of the diet DM, the present study demonstrates the negative relationship associated with feeding over 40% DDGS. Contradictory to the present study Zinn et al. (1997) demonstrated that dietary S concentrations of 0.25% S (S as ammonium sulfate; DM basis) decreased performance and carcass merit; whereas, the present study reported no differences in carcass quality.

During the course of this study there were no confirmed cases of PEM even though dietary S concentrations ranged from 0.6 to 0.9% S, which exceeds the recommended maximum tolerable level (0.3% S; NRC, 2005). These results stand in stark contrast to the recommendations of NRC (2005) and previous research (Loza et al., 2010). Loza et al. (2010) reported that 12 steers developed PEM when fed a co-product based ration containing 0.45% S. In the present study, one steer from the HMC with 60% DDGS treatment did exhibit signs of sulfur toxicity (blind staggers, lack of appetite, and lethargy: McDowell, 2000). This steer responded to treatment with thiamine injections (1 g/d thiamin hydrochloride) and recovered completely within 3 d; histological analysis was not conducted thus a diagnosis of PEM cannot be confirmed. Although only one case of PEM was suspected, sub-clinical cases of PEM could be one explanation for the decreased animal performance of those steers fed 60% DDGS. The lack of confirmed clinical PEM incidence points to a need to clearly distinguish between the maximum tolerable level of S and S toxicity within the scientific literature.

The present study along with Neville et al. (2010) and Schauer et al. (2008) have consistently demonstrated that S from DDGS can be fed in excess of maximum tolerable level in both lambs and steers fed high concentrate diets. It is possible the maximum tolerable level of S (NRC, 2005) needs to be reevaluated. In addition, from a practical application standpoint, factors which may alter fermentation such as grain source, digestibility, and rate of adaptation must be considered as variables influencing S toxicity in the ruminant animal and should be considered when formulating high concentrate rations which included DDGS.

Literature Cited

AOAC. 2010. Official Methods of Analysis. 18th ed. Assoc. of Offic. Anal. Chem. Arlington, VA.

Bird, P. R. 1972. Sulphur metabolism and excretion studies in ruminants X. Sulphide toxicity in sheep. Aust. J. Biol. Sci. 25:1073-1085.

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

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

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.

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

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

Kandylis, K. 1984. Toxicology of sulfur 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 by-products in the beef cattle feeding industry. J. Anim. Sci. 86:1223-1231.

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. Effect of corn processing method in diets containing sorghum wet distillers grains plus solubles on performance and carcass characteristics of finishing beef cattle and on in vitro fermentation of diets. J. Anim. Sci. 87:2124-2132.

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.

Loza, P. L., C. D. Buckner, K. J. Vander Pol, G. E. Erickson, T. J. Klophenstein, and R. A. Stock. 2010. Effect of feeding combinations of wet distillers grains and wet corn gluten feed to feedlot cattle. J. Anim. Sci. 88:1061-1072.

May, M. L., 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 twenty-five percent dried distillers grains on ruminal fermentation and apparent total tract digestion. J. Anim. Sci. 87:3630-3638.

McDowell, L. R. 2000. Vitamins in animal and human nutrition. 2nd Edition. Iowa State University Press. Ames, IA.

Morck, D. W., J. K. Merrill, B. E. Thorlakson, M. E. Olson, L. V. Tonkinson, and J. W. Costerton. 1993. Prophylactic efficacy of tilmicosin for bovine respiratory tract disease. J. Am. Cet. Med. Assoc. 202:273-277.

Nagaraja T. G., and M. M. Chengappa. 1998. Liver abscesses in feedlot cattle: A review. J. Anim. Sci. 76:287-298.

National Research Council. 2005. Mineral tolerances of animals, Second revised edition. The National Academies Press. Washington, D.C.

National Research Council. 2000. Nutrient requirements of beef cattle. The National Academies Press. Washington, D.C.

Neville, B. W., C. S. Schauer, K. Karges, M. L. Gibson, M. M. Thompson, L. A. Kirschten, N. W. Dyer, P. T. Berg, and G. P. Lardy. 2010. Effect of thiamine supplementation on animal health, feedlot performance, carcass characteristics, and ruminal hydrogen sulfide concentrations in lambs fed diets based on 60% DDGS. J. Anim. Sci. 88:2444-2455.

Niles, G. A., S. Morgan, W. C. Edwards, and D. Lalman. 2002. Effects of dietary sulfur concentrations on the incidence and pathology of polioencephalomalacia in weaned beef calves. Vet. Human Toxicol. 44(2):70-72.

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.

Schauer, C. S., M. M. Stamm, T. D. Maddock, and P. B. Berg. 2008. Feeding 60% of lamb finishing rations as dried distillers grains with solubles results in acceptable performance and carcass quality. Sheep and Goat Res. J. 23:15-19.

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

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.

Vasconcelos, J. T., and M. L. Galyean. 2008. ASAS Centennial Paper: Contributions in the Journal of Animal Science to understanding cattle metabolic and digestive disorders. J. Anim. Sci. 86:1711-1721.

Zinn, R. A., E. Alvarez, M. Mendez, M. Montaño, 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.