DISSERTATION

SULFUR METABOLISM IN BEEF CATTLE AND MANAGEMENT STRATEGIES TO IMPROVE PERFORMANCE AND HEALTH IN NEWLY WEANED BEEF CATTLE.

Submitted by:

Kelley L. Neuhold

Department of Animal Sciences

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Fall 2012

Doctoral Committee:

Advisor: John Wagner
Co-Advisor: Terry Engle

R. Kraig Peel
Benard Rollin

ABSTRACT

SULFUR METABOLISM IN BEEF CATTLE AND MANAGEMENT STRATEGIES TO IMPROVE PERFORMANCE AND HEALTH IN NEWLY WEANED BEEF CATTLE.

Five experiments were conducted at Colorado State University's Research Centers. Three experiments were conducted to evaluate sulfur metabolism in beef cattle. The final 2 experiments were conducted to evaluate receiving strategies to improve feedlot performance and cattle health in newly weaned beef cattle.

In experiment 1, rumen fluid from fistulated steers receiving a high roughage (**ROU**; 50% alfalfa hay, 50% corn silage) or a high concentrate-based diet (**CON**; 70% rolled corn, and 30% corn silage) was utilized to examine *in vitro* hydrogen sulfide (H_2S) production of common beef cattle feedstuffs (**FS**). Rumen fluid was collected and combined in equal amounts from 2 rumen fistulated steers that had *ad libitum* access to ROU and mixed at a 3 to 1 ratio of artificial saliva to rumen fluid. Fermentation substrates included: corn, alfalfa, distiller's soluble, dried distiller's grains (**DDG**), and wet distillers grain (**WDG**). Individual substrates (700 mg) were added to separate 125 mL glass serum bottles (in triplicate) with 50 mL of rumen fluid-artificial saliva mixture. Fermentation bottles were capped with an air-tight rubber stopper and incubated in a water bath for 24 h at 39°C. After 24 h of incubation, the total volume of gas produced was measured and a 5 mL gas sample was obtained. Gas samples were then analyzed for H_2S concentration and after gas sampling, pH and dry matter disappearance (**DMD**) were determined. This experiment was then repeated using rumen fluid from steers fed CON. A FS by rumen fluid type (ROU vs. CON) interaction ($P < 0.001$) was detected for μmol of H_2S produced per mg of DMD. Distiller's soluble substrate produced less H_2S per mg of DMD when incubated with

CON compared to ROU rumen fluid while corn, alfalfa, DDG and WDG produced greater μmol of H_2S per DMD when incubated in CON compared to ROU rumen fluid. Across diets (ROU vs. CON), fermentation of corn produced the lowest pH and fermentation of alfalfa produced the highest pH; however, across FS, ROU had a higher pH when compared to the CON rumen fluid. These data suggest that type of rumen fluid (ROU vs. CON) and FS can influence the production of H_2S. Understanding factors that influence H_2S production within the rumen may be useful when formulating beef cattle diets.

In experiment 2, two rumen fistulated cross bred steers were fed ROU for a minimum of 21 d prior to experiment initiation. Samples were prepared for *in situ* digestibility determination by first drying in a 60°C oven for 48 h and then grinding through a 2 mm screen. *In situ* bags were incubated in 2 rumen fistulated steers for 0, 12, 24, or 36 h, removed from the rumen, hand washed, dried in a 60°C forced air oven for 48 h, weighed, and DMD was calculated. Samples were then sent to a commercial laboratory for S analysis. Bags containing a sample from each FS that were washed but not fermented were also sent for analysis. Bags that contained only a minimal amount of the sample were composited with other bags of similar type and time to allow for sulfur analysis. A sample from the original FS was sent for analysis each time samples were sent to the commercial laboratory to determine initial S concentration for each FS. Steers were switched to the CON diet for 21 d and then *in situ* fermentation was initiated again. Data were analyzed as a complete randomized design using PROC MIXED procedures of SAS. *In situ* bag was used as experimental unit for all data analyzed.

The original S concentration of the FS were not statistically analyzed 0.12, 0.34, 0.70, and 0.77 (corn, alfalfa, WDG, and DDG, respectively). Over a 36 h period, DMD was greater ($P < 0.0001$) for FS in the steers consuming the ROU diet when compared to steers consuming the

CON diet. Corn had the greatest ($P < 0.0001$) percent of DMD in ROU and CON diets and alfalfa had the lowest percentage of DMD in roughage and concentrate diets ($P < 0.0001$; 94.71, 83.44, 77.32, and 58.70% ± 2.72; respectively). For the CON diet, corn had the greatest percent S lost followed by alfalfa, then WDG, and finally DDG (67.04, 61.86, 61.39, and 60.13% ± 12.527; respectively). However, in for the ROU diet, alfalfa had the greatest percent S lost followed by WDG, then corn, and finally DDG (89.24, 74.23, 73.46, and 61.48% ± 12.527; respectively). A 4-way interaction ($P < 0.05$) was detected between diet, FS, steer, and time for percentage S loss. This interaction cannot readily be explained; however, this interaction suggests that the S loss from a FS, expressed as percentage of total S, is dependent upon the specific combination of diet, FS, steer, and time being examined and therefore may be of limited value to gauge potential PEM problems. No 3- or 4-way interactions were found when S loss per unit DMD was analyzed. The main effect of FS resulted in DDG having the greatest ($P < 0.0001$) S released per unit DMD and corn released the least, in CON and ROU diets (2.63 and 4.22 vs. 1.15 and 1.03 mg/g ± 0.566) averaged over all time periods. These data suggest that there may be differences in the release of S from different FS in different diets. This finding may be due to the ratio of S types in a FS (i.e. organic to inorganic) or nature of the microbial population associated with the fermentation of CON vs. ROU diets.

In experiment 3, Crossbred yearling steers (n = 432) were used to study the effects of Laidlomycin and Chlortetracycline (**LC**) vs. Monensin and Tylosin (**MT**) and variation in S intake on rumen fluid pH and rumen gas hydrogen sulfide (**H_2S**) concentration. An unbalanced randomized block design using a 2 × 2 factorial arrangement of treatments was utilized. Factors included feed additive (LC vs. MT) and S concentration (constant vs. variable). The variable concentration (**VAR**) was intended to simulate the use of random loads of WDG. Random

numbers were generated for each d of the experiment. High S diets (S = 0.60% of DM) were fed to VAR on d associated with an even number. Low S diets (S = 0.48% of DM) were fed to the constant S treatment (**CON**) all d of the experiment and to the VAR only on d associated with an odd number. From d 0 through 35, a high S dry meal supplement was fed to VAR on the appropriate d. Since variation in S concentration in WDG is driven by rate of inclusion and S concentration in distillers solubles (**DS**), 2 DS based liquid supplements (low S, 0.99% vs. high S, 2.35%) were used to create the CON vs. VAR S intake from d 36 through slaughter. Sulfuric acid was added to the high S DS used to obtain the intended dietary S concentration. On d 35, 70, and 105 rumen fluid and gas cap samples were obtained via rumenocentesis from a subsample (3 hd/pen and 3 pens/treatment) of steers to determine rumen fluid pH and H_2S concentration. The effects of feed additive, dietary S, or the interaction on rumen fluid pH were not significant ($P > 0.38$). An interaction between feed additive and dietary S treatment ($P < 0.02$) existed suggesting that the effect of feed additive on H_2S concentration was influenced by dietary S. Steers fed the CON diet receiving MT exhibited lower H_2S concentration than steers fed LC (1053 vs. 2519 mg/L). Steers fed the VAR diet receiving MT exhibited a higher H_2S concentration than steers fed LC (2567 vs. 2187 mg/L). Rumen H_2S concentration was related to rumen fluid pH ($R^2 = 0.09$) suggesting that management of rumen pH may likely be important in dietary S management.

In experiment 4, 442 newly weaned Angus and Angus crossbred steers (initial BW = 234 ± 40.4 kg) were selected from an initial group of 453 from 3 ranches in CO. These steers were used to evaluate the effect of implant timing on feedlot performance and health. This experiment was conducted as a randomized complete block design. When appropriate, response variables were analyzed on both an individual steer basis and a pen basis. Treatments including an

implant (Revalor®-XS) on d 0 of the experiment (arrival, **ARR**) and the same implant 28 d later (delayed, **DEL**). Both treatments received a common diet and diet changes were made on the same d for both treatments. Live BW was recorded on d 0, 28, 56, 112, 169 and prior to harvest. On d 0, 5 steers from each pen were vaccinated with 2 mL of ovalbumin (**OVA**) subcutaneously and 1 mL was administered intradermally. On d 28 the same 5 steers received a booster of the OVA and 5 novel steers also receive a vaccination of OVA. This allowed a d 0 and d 28 primary responses to be evaluated along with a d 28 secondary immune response.

On an individual steer basis, ARR steers had a tendency ($P < 0.07$) for heavier BW then DEL steers on d 28 (293 vs. 291 ± 1.7 kg, respectively). Likewise ADG for d 0 through 28 favored the ARR treatment over the DEL (1.80 vs. 1.71 ± 0.056 kg, respectively). However, by d 56 BW were again similar ($P > 0.11$). The final BW for the DEL treatment were greater ($P < 0.05$) as compared with the ARR treatment (636 vs. 627 ± 8.3 kg, respectively). This increased final BW is consistent with the overall ADG where the DEL treatment out gained ARR (1.78 vs. 1.74 ± 0.029 kg, respectively). Steers in the ARR treatment had increased DMI over the DEL treatment for d 0 through 28, d 29 through 56, and d57 through 112 ($P < 0.018$). However, from d 113 through 169 and d 170 through harvest there was no difference ($P > 0.20$) between treatments. Therefore, total DMI was greatest ($P < 0.06$) for the ARR treatment when compared to the DEL (9.81 vs. 9.56 ± 0.194 kg, respectively). Arrival steers had a lower gain to feed (0.175 vs. 0.186 ± 0.0033) and a higher feed to gain ratio (5.72 vs. 5.40 ± 0.101) when compared to DEL steers ($P < 0.0001$). Hot carcass weight was different ($P < 0.0001$) with the DEL have a 6 kg heavier weight the ARR (384 vs. 378 ± 4.7 kg, respectively). Carcass quality grade, yield grade, dressing percentage, and longissimus dorsi area were not different between treatments ($P > 0.4$). No differences were found between DEL and ARR treatment for cattle that were treated

1, 2 or 3 times during the feeding period ($P > 0.16$). Immune response to the OVA injection that was given at d 0 and d 28 tended to be different ($P < 0.07$) with the ARR steers having a higher concentration OVA IgG. In steers vaccinated on d 28 which had not received OVA on d 0 no difference was found between DEL and ARR treatments ($P > 0.49$). These data suggest that delayed implantation did not impact health or immune response. However, under the conditions of this experiment delaying implanting 28 d did improve overall feed efficacy.

In experiment 5, 124 newly weaned Angus, Hereford, and Angus × Hereford bull and heifer calves (initial BW = 233 ± 14.9 kg) were utilized to evaluate 2 feedlot receiving management strategies at Colorado State University's Agricultural Research, Development, and Education Center in Fort Collins, CO on feedlot performance over the first 30 d upon arrival to the feedlot. Cattle were blocked by gender and stratified by BW, breed, and age, and assigned to 1 of 14 pens (8 - 10 head/pen). Pens were then assigned to 1 of 2 dietary treatments. Dietary treatments included: 1) a dried distiller's grain-based total mixed ration (**DDG**) initiated upon arrival, or 2) long-stem grass hay followed by a total mixed ration containing no DDG (**HAY**). Calves receiving the HAY treatment received only grass hay for the first d after arrival, long stem grass hay and total mixed ration combination the following 2 d, followed by a grain based total mixed ration on d 4. Beginning on d 4, calves across all treatments had access to iso-caloric and iso-nitrogenous diets. Calves were weighed on d 0 and 30, and DMI was determined daily. Initial BW was similar ($P = 0.99$) across treatments; however, d 30 BW was greater ($P < 0.001$) for DDG vs. HAY calves. As a result, ADG was greater ($P < 0.001$) for DDG vs. HAY calves (0.59 vs. 0.41 ± 0.04 kg/d, respectively). Gain-to-feed ratio was greater ($P < 0.05$) for DDG vs. HAY calves (0.22 vs. 0.17± 0.013, respectively), and feed-to-gain ratio tended ($P = 0.05$) to be greater in HAY vs. DDG calves. Daily DMI tended ($P = 0.06$) to be greater in DDG vs. HAY

calves (2.70 kg·hd^{-1}·d^{-1} vs. 2.35 kg·hd^{-1}·d^{-1} ± 0.256, respectively). In summary, providing a DDG-based receiving ration to newly weaned calves upon arrival to the feedlot resulted in greater feed intake, gain, and feed efficiency over a 30 d period than traditional long-stemmed grass hay followed by a non-DDG total mixed ration.

Key words: Cattle, hydrogen sulfide, performance, receiving, sulfur

ACKNOWLEDGMENTS

This dissertation is dedicated to Mrs. O'Brian, who called me Mr. Neuhold in the hall at high school one day and I turned to her and said no that's Dr. Neuhold. She quickly replied I will never call you that you're not that smart. She was probably right but I was determined enough!

I would also like to dedicate this to Joe and Linda Neuhold. They always made me think I could do anything I wanted to, even if they didn't think I should.

I would like to take this chance to say thank you to all of the people who helped me though this process. I would like to start by thanking the four gentlemen on my graduate committee John Wagner, Terry Engle, Kraig Peel, and Bernie Rollin. I have learned so much from all of you. I would also like to thank ADM Alliance Nutrition Loomix for hiring me and allowing me to start working with the flexibility to finish my dissertation.

I would like to thank my Mom and Dad I owe you two more than I could ever repay. You two have been my anchors and my best friends. Thanks for helping me with whatever I need. I couldn't have asked for better parents.

I would like to thank all the graduate students that helped me with research and befriending me over the 5 years of graduate school. There are too many to name you all but I few that I would like to recognize would be Kurt Vogel, Jenny Shultz, Liz Domby, Ryan Peterson, Elin Westover, Paul Repenning, Emmanuel Caldera, Bo Bigler and Cari Jo Berrett. You guys really made graduate school bearable. I would also like to think Drs. Archibeque and Ahola I enjoyed working with you two and getting to know you both. A big thanks to the crews at the 3 research centers where I conducted these experiments. Also a special thanks to Doug Couch, I really enjoyed working with you.

Thanks to Alpharma for sponsoring one of the projects, and Drs. Ahola, Engle and Wagner for finding funds to conduct the other projects.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

REVIEW OF LITERAURE

Sulfur is an essential element in the diets for all mammals (NRC, 2005). Ruminants are more susceptible to S toxicity than non-ruminants. Excess S intake in ruminants can cause blindness, lethargy, diarrhea, seizures, head pressing, anorexia, and death (Coghlin, 1944; Gould, 1998). Initially, S was considered a nontoxic mineral; however, in 1956 S toxicity was documented to cause polioencephalomalacia (**PEM**) in ruminants (Jensen et al., 1956). Briefly, excess S can be converted by rumen microorganisms into sulfide which binds hydrogen thusly forming hydrogen sulfide gas (**H_2S**). Two main theories have been proposed to explain how excess S induces PEM. Theory 1: H_2S enters the blood stream through the lungs and crosses the blood brain barrier and inhibits cytochrome-C in the electron transport chain thereby impairing the production of ATP in brain cells. Theory 2: sulfide and other thiaminases in the rumen destroy thiamin produced by ruminal microorganisms which induces a metabolic thiamin deficiency within the animal leading to impaired energy metabolism in brain cells.

Many of the early recorded outbreaks of PEM were due to cattle consuming water containing 1000 mg/L or greater sulfate. Monitoring sulfate water sulfate concentrations and limiting access to high sulfate water are the primary management strategies used by beef cattle producers to reduce the incidences of PEM. However, the availability of ethanol co-products for cattle feeding has increased due to an expansion of ethanol production. Unfortunately, ethanol co-products typically have elevated S concentrations. Feeding of ethanol co-products has led to incidences of PEM in locations where PEM has not been documented in the past. Currently the majority of confirmed cases of PEM are in feedlot cattle that are consuming high concentrate

diets. Limited information is known about the true cause of PEM (Goetsch and Owens, 1987) or the factors that affect H_2S production *in vivo*. Furthermore, limited information is available regarding sub-clinical cases of PEM.

Sulfur Metabolism: In a ruminant animal, S can enter the gastrointestinal tract via: 1) feed, 2) water, and/or 3) endogenous secretions (i.e. saliva). Sulfur can enter the animal as either organic forms such as proteins, or inorganic forms such as sulfates. Methionine, cysteine, homocysteine, taurine, cystathionine, cysteic acid, and cystine are all amino acids that contain S (NRC, 2005). The B vitamins, thiamin and biotin, also contain S. National Research Council (**NRC**) describes the S requirement for feedlot cattle as 0.15% of diet dry matter, and the maximum tolerable limit is 0.4 percent of diet dry matter (NRC, 2000).

Two classes of rumen microorganisms, assimilatory and dissimilatory, are responsible for S metabolism in the rumen (Gould, 2000; Underwood and Suttle, 2001). Assimilatory microorganisms reduce S and then incorporate the elemental S into S containing amino acids. Dissimilatory microorganisms use elemental S as an electron acceptor; therefore, the reduced forms of S (sulfite and sulfide) are a metabolic end product of fermentation from these microorganisms (Gould, 2000). Underwood and Suttle (2001) suggested that assimilatory microorganisms only use organic forms of S. Other factors that can affect S metabolism in the rumen are fermentable carbohydrates, nitrogen, pH, and other minerals (Underwood and Suttle, 2001). Some sulfides are absorbed through the rumen wall and H_2S is expelled through eructation. It is hypothesized that majority of the S flows out of the rumen into the omasum in the form of rumen un-degraded protein, microbial protein, or inorganic forms of S. Little is known about S metabolism in the omasum.

Sulfur metabolism is very similar in ruminants and non-ruminants once S enters the abomasum. Inorganic S is absorbed primarily through active transport. Organic forms of S contained within proteins are broken down into S containing amino acids in the small intestine by proteolysis. The amino acids are then transported into the circulatory system via a sodium dependant transport mechanism. The majority of excess absorbed S is excreted in the urine as sulfate.

Polioencephalomalacia: Polioencephalomalacia or cerebrocortical necrosis (Jensen et al., 1956) is a metabolic disease that occurs in ruminant animals. There are many causes of the onset of this disease including mercury poisoning, trauma to the head, selenium toxicity, salt toxicity, lead poising, thiamin deficiency, and S toxicity. Hereafter in this review, PEM will refer to S induced PEM. Polioencephalomalacia is a softening of the gray matter of the brain, similar to the damage that occurs in the brain of a human suffering from a stroke (Gould, 2008). Polioencephalomalacia is only truly diagnosed by an examination of the brain post-mortem (Merck Veterinary Manual, 1998; Gould, 1998). Animals affected by PEM do exhibit clinical signs that indicate PEM such as blindness, lethargy, poorness, seizures, head pressing, circling, anorexia, saw horse stance, and death (Coghlin, 1944; Gould, 1998). The damage to the brain in animals with PEM is permanent. Depending on the severity of the insult, some cattle do survive. If the affected animal does not die, the animal will likely have a reduced feed intake and growth rate.

High sulfate water, high S forages (alfalfa or Canada thistle), corn co-products (wet distiller grain, dry distiller grain, corn gluten feed, and corn distiller's solubles), dried whey, molasses based feeds, fertilizers, and S salts are common beef cattle feed ingredients that can contribute to the S concentration in the diet. Sulfate can also enter the rumen via saliva through

a process called S recycling (Church, 1988). However, the amount of S recycled is very small 1.2 to 2.5 mg/d/kg BW when compared to the S entering the rumen from the diet (Kandylis, 1983).

The majority of the PEM cases have been reported in feedlot and stocker cattle; however, PEM has been reported in most classes of cattle and sheep. It is proposed by many animal scientists that a lower ruminal pH increases the risk of PEM (personal communication). Therefore, it has been hypothesized that roughage-based diets lower the incidence of PEM in the presence of excess S. However recent research has shown that pH explains approximately 12 percent of the variation of ruminal H_2S concentration (Sarturi et al., 2011).

There appears to be an individual animal component to S induced PEM, resulting in varying degrees of severity of clinical signs and some variation in the onset of symptoms during an animal's life. The amount of damage to the brain is also variable. In an experiment conducted at Southeast Colorado Research Center (**SECRC**) in Lamar, CO cattle were fed diets containing well above (0.48% and 0.60%) the recommended maximum of 0.4%total dietary S (dietary S was from both water and feed sources). Field necropsies were preformed on all animals that died during the experiment and brain tissue from each animal was sent to the Colorado State University diagnostic laboratory in Fort Collins, CO. Upon examination of brain tissue, all samples submitted revealed signs consistent with PEM, even in cattle that had shown no clinical signs of PEM and were diagnosed as dying from causes other than PEM (Domby, 2011). If diagnosed soon enough, the symptoms of PEM can be lessened and in some cases alleviated. However, it is unlikely that visual observation alone will be sensitive enough to identify cattle at risk of PEM. It is important to understand how weather, diet mixing, water intake, feed sorting, H_2S production as it relates to feedstuff (**FS**) combinations, and other factors

may be involved in the amount of damage and onset of when PEM symptoms occur will help to reduce the incidence of PEM.

Eructation Theory: One of the most commonly accepted theories as to the cause of PEM is the eructation theory (Olkowski, 1997; Gould, 2008). This theory consists of the ruminant animal consuming a high S diet with the excess S being reduced to sulfide in the rumen. The sulfide is then associated with hydrogen ions to form H_2S. The H_2S gas pools in the gas cap of the rumen along with other gases that are by-products of fermentation. When the gas increases the pressure in the rumen, the gas is eructated through the mouth and/or nose of the animal. Ruminant animals are prey animals and the sound and smell of an eructation could alert a predator to the animal's location. Therefore, ruminants have developed a defense mechanism, which has evolved over time, of inhaling their own eructation (Personal communication, Wagner, 2011). This would cause the H_2S that has built up in the rumen to enter the lungs. Hydrogen sulfide very insoluble therefore it travels deep into the alveoli of the lungs and is readily absorbed (Klaassen, 1996). After entering the blood stream it travels to the brain without passing through the liver where H_2S could be converted to sulfate. Once H_2S reaches the brain, it crosses the blood brain barrier and inhibits cytochrome-C in the electron transport chain. The inhibition of cytochrome-C reduces the ability of the cell to produce ATP (Smith et al., 1977 and Murray et al., 2009) and thus starves the cell of energy and results in cell death which presents as lesions on the brain that fluoresce when examined under ultra violet light.

Thiamin Theory: Another widely excepted theory is that high S consumption causes a thiamin deficiency in the animal (Olkowski, 1997). As rumen microorganisms reduce S from sulfate to sulfide, an intermediate is produced called sulfite. Sulfite destroys thiamin (synthesized by rumen microorganisms) by cleaving the methylene bridge that joins the

pyrimdine ring and the thiazole ring of thiamin (Combs, 1998). This thiaminase activity by sulfite is increased at neutral or acidic pH, thus grain based diets would exacerbate the problem. The drop in ruminal pH due to the consumption of a high concentrate diet also causes a shift in the microorganism population; many of these microorganisms (*Clostidium* spp., *Bacillus* spp., gram negative and gram positive cocci, and gram positive bacilli) have cell surface thiaminases. At low pH, these microorganisms release thiaminase I enzyme which also destroy thiamin in the rumen (Merck Vet. Manual, 1998; Combs, 1998). This culminates in a thiamin deficiency in the animal, impairing glucose metabolism and starving the cells of energy.

Thiamin induced PEM has been experimentally induced (Spicer and Horton, 1981; Fakhruddin, 1987). However, experiments using high S diets to induce a thiamin deficiency and PEM have not been successful. Oliveira et al. (1996) fed a high sulfate - thiamin free diet and was not successful at reducing thiamin blood concentrations even in the presence of elevated ruminal sulfide concentrations. Gould et al. (1991) were also unable to show a reduction in thiamin concentrations in tissue or rumen fluid in animals diagnosed with PEM.

Sulfur Sources: Sulfur can be ingested by an animal in many different forms. Sulfur can be presented to the animal in an inorganic form such as in water as sulfate or organic forms of S such as S containing amino acids. Very seldom do animals ingest elemental S; however, elemental S can be fed for medicinal purposes or added to the diet as a S supplement when S is low in the basil diet. The National Animal Health Monitoring System's (**NAHMS**, 1999) survey reports that 22.6% of the reporting feedlots, have water that contains 300 or greater mg/L of sulfate. National Animal Health Monitoring System considered less than 300 mg/L safe for livestock consumption. NRC (2000) states that a steer weighing 454 kg with an ambient temperature of 14.4° C, has a water requirement of 41 liters per d. If the water contained 300

mg/L the steer would be consuming 12.3 g of sulfate per d and since S makes up one-third the molecular weight of sulfate the steer's S intake would be 4.1 g of S per d. If this animal has a DMI of 9.5 kg per d its S requirement would be 14.25 g of S per d; therefore, daily water intake would account for 29% of its total daily S requirement. If that same steer consumed water containing 1000 mg/L sulfate at an ambient temperature of 26.6°C its S intake would be 18.3 g or 128% of its daily requirement without any S intake from feed.

Sulfur content can vary greatly both within and between FS. Table 1.1., created from the NRC (2000), demonstrates the variability of S content in common FS. The majority of S content of a typical finishing feedlot diet is contributed by the corn, even though corn only contains, on average, 0.11% S. This is due to the fact that corn makes up between 70 to 90% of a typical feedlot ration.

The growth of the ethanol industry has brought about higher corn prices and an abundance of distiller's grains and other ethanol co-products. Many producers have replaced corn in feedlot diets with distiller's grains to try to mitigate high feed cost. The ethanol process removes the starch from the grain and leaves the proteins, fats, and minerals as a by-product. This by-product is known as distiller's grains. Starch makes up about two-thirds of the corn seed and the other one-third remains as a by-product after ethanol production; thus, concentrating the remaining nutrients. Sulfuric acid is also used in the cleaning of ethanol plants and to manipulate the pH of the mash during fermentation (Ensley, 2011). This can end up in the distiller's grains and add to the S content of the by-product. For these 2 reasons the S content of distiller's grains greatly exceeds the original S concentration of the 0.11% of the corn used to make the ethanol. Therefore, replacing corn with distiller's grains in feedlot diets can cause the S intake of feedlot cattle to be elevated. Table 1.2. demonstrates the effect of the replacing corn

with distiller's grains in a typical feedlot finishing ration and the addition of high sulfate water on the S intake of a feedlot steer. This table demonstrates that the requirement of 0 .15% (NRC, 2000) and that the maximum tolerable S level of 0.4% (NRC, 2000) can be rapidly exceeded with total combination of distiller's grains and water sulfate.

Recently the University of Nebraska and Iowa State University hosted a webinar (January, 2011) on the subject of S concentrations in feedlot diets. This webinar questioned whether the maximum concentration set by the NRC (2000) is appropriate or if this limit should be increased. At the end of the webinar presentation it was suggested that the maximum limit should be increased to 0.45% based on the data presented. Other work from the University of Nebraska suggests that not only the total concentration of S should be considered but also the type of S. Sarturi et al. (2011) suggests there are 2 types of S in a ruminant diet; rumen degradable S and rumen un-degradable S, thus suggesting the source and type of S should be considered when formulating diets.

Scope of S Exposure: Production losses are hard to quantify due to the fact that not all PEM cases are reported and producers may not know if a reduction in feed intake is due to a change in S in the FS unless it is closely monitored. However, it is possible to try to estimate potential production losses from the published research. Cattlefax reported that in 2010 the USA harvested over 27 million head of cattle. Current projections have those numbers falling due to a shrinking of the USA cow herd. For the sake of this paper we will use this number, 27 million, to estimate possible exposure to S toxicity.

From NAHMS (1999) survey we know that 22.6 percent of the feedlots provide cattle with water that has 300 mg/L or greater sulfate water, thus exposing them to potential S toxicity. Using this number we can estimate that of the 27 million cattle 6.1 million cattle are exposed

annually to high sulfate water. Water is not the only possibility of S exposure; distiller's grains have also become a concern as well. According to the Renewable Fuels Association in 2010 32.5 million metric tons of distiller's grains were produced, and they estimate that 80% of those grains were consumed by cattle. However, only 13.3 million metric tons were fed to beef cattle. Assuming that the average steer consumes 10 kg of DM and is fed a ration containing 40% distiller's grains, there would be a rate of 4 kg per animal per d consumed. This would mean that 9.1 million head of beef cattle per year could be exposed to S toxicity from distiller's grains annually. This means that 15.2 million cattle annually or half the cattle harvested in the United States could be exposed to S toxicity from water and distillers grains. Other by-products and FS can add to this problem to make it a real concern.

Production Losses: Production losses due to S toxicity in the feedlot can range from a decrease in DMI and ADG to death. Loneragan et al. (2001) used a range water sulfate concentrations (control < 140 mg/L sulfate; maximum >2000 mg/L sulfate) to study S toxicity in feedlot cattle. They found that excess S consumption reduced ADG, DMI, longissimus area, and dressing percentage. In turn it increased days on feed and reduced feed conversion. When comparing the control to the animals receiving > 2000 mg/L sulfate, ADG was reduced by 0.10 kg per d. Gain to feed was reduced 0.02 kg/kg for every 100 mL increase in the concentration of sulfate in the water. The animals receiving > 2000 mg/L sulfate also had a reduction in water intake over the entire feeding period when compared to the control (30.1 vs. 34.4 L/d, respectively). It is unclear how much of the reduction in performance is due directly to S toxicity and how much is due to the reduction in water intake.

Mortality and morbidity rates can greatly affect the profitability of a cattle operation. An experiment performed at SECRC used a combination of water sulfate and distiller's grains to

induce S toxicity. This experiment, reported morbidity was 1.8% of the cattle and 2.1% died or were removed from the trial due to PEM (Domby, 2011). Merck Veterinary Manual (1998) reports morbidity rates as high as 19% due to S toxicity. Merck also reports a 50% or greater mortality rate of untreated animals suffering from PEM.

The USDA market reports that on January 10th, 2011 steers averaging 330 kg in La Junta, CO sold for an average of $1093 per steer. Assuming that 15.2 million head of cattle are exposed to high sulfate, and a 2% death loss among these cattle, a total loss of 304,000 steers at cost of $332 million annually can be estimated. This estimate does not include losses due to increased days on feed, treatment cost, or losses in carcass quality due to S toxicity.

Current Treatments: The efficacy of treating animals with PEM is mixed. First the source of excess S should be removed from the animals diet (Gould, 2000). The majority of treatments include the use of thiamin or thiamin yielding products. If the animal is suffering from thiamin deficiency this could and should work if administered in a timely fashion. However, if the PEM is induced by H_2S poisoning, treating the animal with thiamin will likely not be helpful. The mixed results may be due to the fact that both types of cases could be occurring in the same group of animals; therefore, what helps one may not help the other.

Merck Veterinary Manual (1998) suggests treating PEM with injections of thiamin hydrochloride the rate of 10-15 mg per dose for up to 3 d. The manual also suggests administration of furosemide up to 1 mg/kg of body weight to help with cerebral edema and dexamethasone at a rate 1-2 mg/kg of body weight. It is also common practice by some producers (personal communication) to add thiamin to the ration of cattle at risk of developing PEM. National Research Council (1984) reports that feeding supplemental thiamin to feedlot cattle has had mixed results in reducing the incidence of PEM. If the PEM is the result of a

thiamin deficiency caused by thaminases in the rumen, oral thiamin would be exposed to destruction by thiaminases, and the amount that would need to be fed to overcome the thiaminases activity would likely be cost prohibitive.

The administration of thiamin appears to be the most often prescribed and successful treatment for PEM. This could lead one to believe that PEM is related to a thiamin deficiency. However, Olkowski (1997) suggest that thiamin may act as an antioxidant in the brain and help to reduce damage cause by excess sulfide.

Stress: Many authors have tried to define stress and many more use the word without definition. For use of the term in this paper, stress is defined as a nonspecific external or internal force acting on an animal that causes the animal to deviate from homeostasis thereby negatively impacting performance (i.e. ADG, milk production, or egg production). Grandin (1997) states that there are 2 types of stress: psychological and physical. Psychological stressors include but are not limited to human interaction, novelty, and physical restraint in a squeeze chute. Physical stressors include but are not limited to standing on a truck, lack of food and water, excess intake of a single nutrient (i.e. S), and ambient temperature (Grandin, 1997). Weaning and receiving cattle into a feedlot are 2 of the most stressful times in a calf's life and for many calves these 2 events happen at or near the same time. A calf that is being weaned into a dry-lot system will most likely be exposed to all of the aforementioned stressors and many more. National Research Council (2000) suggests there are 2 components to managing stress in cattle: 1) managing the inducers of the stress and/or 2) managing the result of the stress. Stressors associated with handling cattle can be reduced by decreasing the frequency of handling, keeping noise down when handling, and only using cattle prods when needed. In open feedlots, like most of the feedlots in the western United States, weather cannot be managed; however, we can try to

minimize the stressors of inclement weather by using bedding, increasing water trough space, and nutritional manipulations. These strategies may help the animals to perform better during stressful events and recover faster after the weather event. Stress and nutrition are interrelated and both must be considered when developing management strategies to receiving highly stressed calves into a feedlot (Hutcheson and Cole, 1986).

Stress Health Interactions: Duff and Galyean (2007) suggest that transportation, marketing, and commingling all have a negative impact on calf immunity. They also suggest that temperament of the animal can influence immunity. Temperament is the animal's tolerance and ability to cope with psychological stress. Hutcheson and Cole (1980) state that environmental stressors affect morbid more than healthy calves, suggesting that stressors may have an additive negative impact on feedlot performance. Acute phase protein concentrations in the blood of cattle increase in response to stress (Conner et al., 1988). The acute phase proteins are produced after stimulation from pro-inflammatory cytokines. Pro-inflammatory cytokines have been shown to inhibit growth and increases proteolysis in animals (Johnson, 1997). Activating this immune response for the production of the acute phase proteins increases the animal's demand for nutrients specifically protein and to replace lost tissues (Arthington et al., 2005).

Implants: The cattle industry has commonly used growth promoting implants for over 55 years to improve ADG and feed conversion (Belk, 1989 and Hancock et al., 1991). Improvements of 6, 15, and 20% in ADG can be realized in calves, stockers, and feedlot cattle, respectively when implanted with a growth promotant (Duckett and Andrae, 2001). Implanting in every phase of beef cattle production (suckling, stocker, and feedlot would increase value by approximately $93/head (Duckett and Andrae, 2001). Griffin et al. (2009) investigated the

effects of delayed implanting on feedlot performance and carcass merit. They reported no difference between the 2 treatments of implanting on arrival into the feedlot or delayed implanting until 30 d after arrival in the feedlot. However, Griffin et al. (2009) suggest that more research is need on strategies for implanting high risk cattle. Duff and Galyean (2007) question the effect that implants have on the immune system of highly stressed calves (Figure 1.1).

Samber et al. (1996) compared 7 implant treatments, a negative control that received no implant, 2 treatments the received Ralgro® upon arrival at the feedlot, 2 treatments that delayed first implant until d 30 on feed and 2 treatments that receive Revalor-S® upon arrival at the feedlot. Samber et al. (1996) did not find a difference in ADG, G/F, or live BW between steers implanted upon arrival and the steers that were delayed implanted on d 30. Steers that were not implanted did have a lighter final live BW and lower ADG over the entire trial when compared to steers that were implanted; however, health data were not reported from this experiment which could give insight into the effect of delayed implanting on health in feedlot cattle. If delayed implanting does not negatively impact feedlot performance and reduces morbidity, delayed implanting could be a very economical solution for receiving high stressed calves. Bruns et al. (2005) compared 3 treatments: no implant, implant on arrival at the feedlot, and delayed implant at d 56. They found a improvement in live BW, ADG, and G:F in the cattle implanted upon arrival in the first 56 d when compared to the other treatments. However, on d 112 there was no difference between the delayed treatment and the implanted upon arrival treatment for live BW but ADG and G:F favored the delayed treatment. On d 140 there were no differences between the 2 implanted treatments for feedlot performance. Again, no health data was reported for this experiment. Both Bruns et al. (2005) and Samber et al. (1996) reported no difference in DMI

between control cattle that received no implant and cattle that received an implant suggesting the in the increase in feed conversion is due solely to improvements in ADG.

Tillman and Brethour (1957) conducted an experiment with a 2 x 2 factorial arrangement of treatments; factors included level of protein and with or without diethylstilbestrol (**DES**). The 2 levels of protein fed to the lambs were 6 and 10% and lambs that received DES received an oral 3 mg dose. They reported that lambs receiving an oral dose of DES and a diet containing 6% protein had decreased gain when compared to the lambs receiving the same diet and not receiving DES; however, the lambs receiving DES on the 10% protein diet had greater gains then those on the same diet not receiving DES. Cole and Todd (2008) preformed a review of research of ways to enhance performance and efficacy through synchronizing nutrients in grain fed ruminant diets and found that oscillating protein in some instances decrease performance in feedlot cattle. They attributed this finding to the aggressiveness of the implants used. Rumsey et al. (1973) evaluated Angus steers with and without DES fed a 75% concentrate diet *ad libitum* for 28 d, then restricted intake to 0.9 kg per head per day for 35 d, and then fed *ad libitum* for 60 d. They reported that steers that received DES had an increased ADG when compared to the non-DES steers in the first and last period (1.32 vs. 0.92 and 1.74 vs.1.34 ADG (kg), respectively). In the restricted period animals across all treatments lost weight however, steers that received DES lost more weight than steers that did not receive DES (-1.55 and -1.22 ADG (kg), respectively). This is consistent with what Tillman and Brethour (1957) found in lambs suggesting that giving animals a growth promotant when nutrient intake is restricted can have negative effects on growth. Research has shown that hormonal growth promotants increase basal metabolic rate and heart rate; they have also been shown to effect nitrogen excretion (Rumsey et al., 1973; 1980; Rumsey and Hammond, 1990). Rumsey et al. (1981) and Rumsey (1982)

showed that steers implanted in the feedlot increased their protein deposition by more than 20% when compared to non-implanted steers. This would indicate that nitrogen intake becomes even more crucial for implanted cattle.

Nutrition: Stress and nutrition are interrelated; stress can exacerbate any nutritional deficiencies and nutritional deficiencies can initiate a stress response in an animal (NRC, 2000). Nutritional strategies can affect calf performance, morbidity, and mortality (Hutcheson and Cole, 1986). Cattle treated for respiratory disease have lower ADG, HCW, and lower quality grades (Gardner, 1999). During the first 1 to 3 weeks in a feedlot, calves have depressed feed intake (Hutcheson, 1980). After reviewing 18 experiments, Hutcheson and Cole (1986) found that not all calves eat during the first 2 weeks in a feedlot. Sowell et al. (1998) found a 30% decrease in time at feed bunk for morbid calves, and this was most pronounced in the first 4 days after arrival. Therefore getting calves to eat as soon as possible could reduce morbidity, animal well-being, improve feedlot performance, and profitability of the cattle.

Increasing energy demand for growth through the use of growth promotants coupled with a reduction in feed intake in newly received calves would warrant feeding a high concentrate diet. However, NRC (2000) suggests that receiving diets for highly stressed calves should not exceed 25% concentrate and 4% fat. If a high concentrate diet is used supplementation of hay for the first 3 to 7 days could be used to of set the negative impacts associated with a high concentrate diet (NRC, 2000).

With increased supply of ethanol by-products we see increase in the concentration of them used in cattle diets. It is wildly believed that novel FS reduce intake; however, distiller's grains appear to be highly palatable. They have a high energy content and a high fiber content (1.50 Mcal/kg and 46.0 % NDF; NRC, 2000). There are 2 down sides to using distiller's grains

in starting diets, a great percent of the energy comes from fat (9.8%) and they have a high S (0.40%) concentration (NRC, 2000). If the other diet ingredients and the source of water are not high in S, S should not be an issue.

Conclusion: The evidence linking PEM to sulfur toxicity is overwhelming. However, the mode of action and treatments for this disease is still under debate. More information is needed to understand S metabolism which will help both with understanding mode of action and help to treat the disease. Currently total S in the only measurement used to balance ration for cattle despite some evidence that S source have differing availabilities *in vitro*, therefore more information is needed to enable nutritionist to consider source of S. Polioencephalomalacia is highly variable between animals; more information is needed to understand factors that contribute to H_2S production in the rumen such as, ruminal pH, S source, microbial populations, and mode(s) of action.

The stress associated with transportation and feedlot arrival, cause calves to become very susceptible to disease. Steps should be taken to reduce the stress experienced; however, factors such as transportation time, fasting, weather, and comingling may not be able to be avoided. Mitigating the effects of these stresses should be considered. Due to the increased demand in the body for protein and energy created by growth promotant implants and the need for protein and energy to mount an immune response cattle that are arriving into a feedlot may have difficulty remaining healthy. Add on top of this increased demand for energy and protein the reduction of feed intake by calves the first 3 weeks in the feedlot and we have a situation ripe for disease. Delaying implanting may be one step that can be taken to mitigate some of the health issues associated with arrival at the feedlot. The evidence shows little to no difference when delaying

implanting 2 to 4 weeks after arrival and little work has been published on the effect delaying has on the health of calves.

With the increased stress and exposure to disease agents upon arrival in the feedlot, and the reduction of feed intake in the first 3 weeks of arrival into the feed yard it is very important that receiving diets are formulated to encourage as much feed intake as possible. Also, diets must be energy dense to ensure that every kg of feed consumed contains the appropriate amount of energy and protein for that classification of animal. With all of the new by-products available to producers, these products must be evaluated to as to their ability to draw calves to the bunk and promote feed intake.

Table 1.1. Sulfur concentration in common feedstuffs.

Feedstuff	Sulfur, %	SD ±
Cracked Corn	0.11	0.02
Corn Silage	0.12	0.03
Alfalfa Hay	0.28	0.03
Distiller Grains Plus Solubles	0.44	0.12
Soy Bean Meal	0.46	0.06
Molasses	0.60	0.05

Table 1.2. The increase of sulfur concentrations as a percent of a ration with increasing levels of distiller's grains and water sulfate.

Items[b]	0	300	1000	1500
Finishing diet 0% DG[a]	0.15	0.197	0.309	0.390
Finishing diet 10% DG	0.17	0.217	0.329	0.410
Finishing diet 20% DG	0.19	0.237	0.349	0.430
Finishing diet 30% DG	0.22	0.267	0.379	0.460
Finishing diet 30% DG at .56% S	0.26	0.307	0.419	0.500
Finishing diet 40% DG	0.25	0.297	0.409	0.490
Finishing diet 40% DG at .56% S	0.30	0.347	0.459	0.540

[a] Based on 48 L/d of water intake and 10 kg/d of DMI.

[b] Based on a finishing diet containing flaked corn, corn silage, alfalfa hay, and soybean meal. Ration was formulated to provide a NEg of 65 Mcal/45.36 kg DM and 13.5% CP.

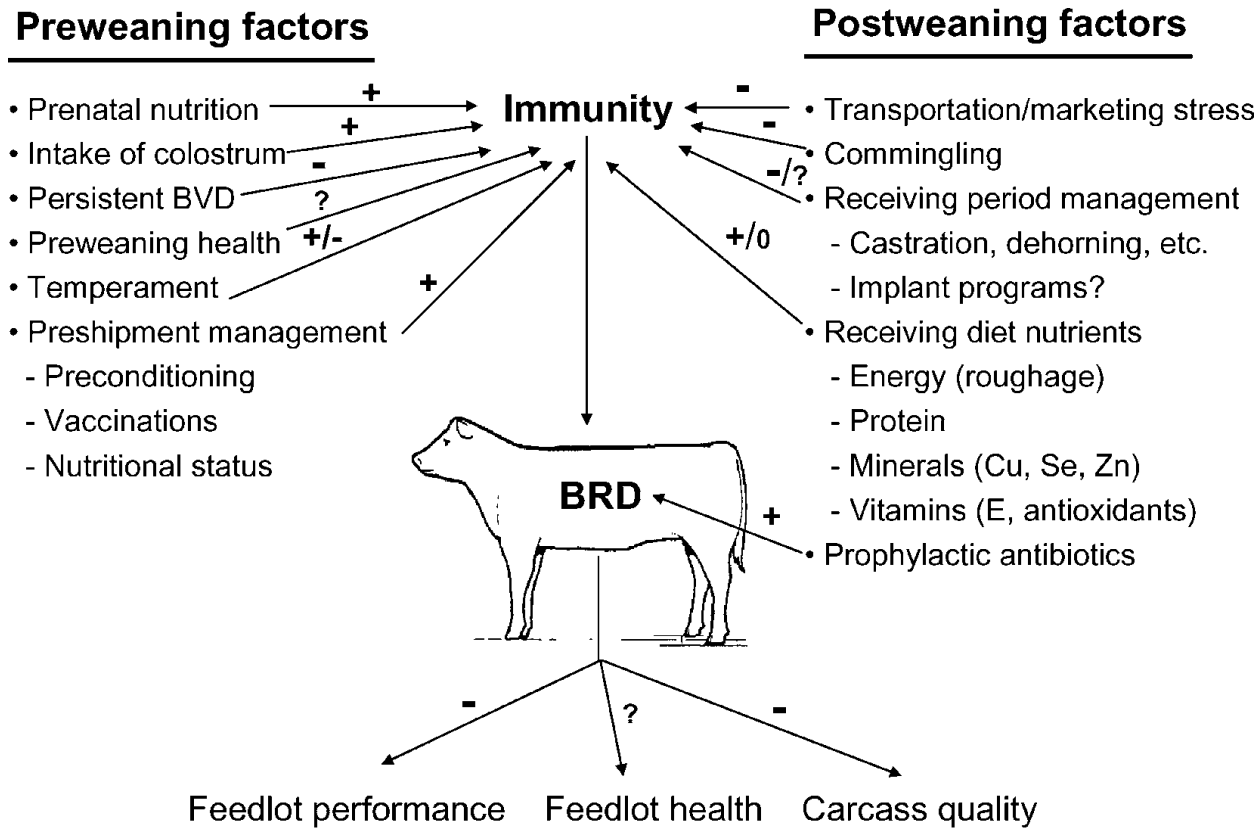

Figure 1.1. Pre- and postweaning factors affecting bovine respiratory disease in beef cattle and the resulting outcomes of the disease. (Duff and Galyean, 2007).[1]

[1]+ = decreased incidence or consequence; − = increased incidence or consequence; ? = effects not fully understood based on the available data. BVD = bovine viral diarrhea virus.

LITERATURE CITED

Arthington, J. D., J. W. Spears, and D. C. Miller. 2005. The effects of early weaning on feedlot performance and measures of stress in beef calves. J. Anim. Sci. 83:933-939.

Belk, K. E., H. R. Cross, and D. Nash. 1989. Effects of trenbolone acetate, estrogens on steer explored. Feedstuffs 61(50):15.

Bruns, K. W., R. H. Pritchard, and D. L. Boggs. 2005. The effect of stage of growth implant exposure on performance and carcass composition in steers. J. Anim. Sci. 83:108-116.

Cattlefax. 2012. http://www.cattlefax.com/page_attachments/0000/9144/WEB_LTO_12__15_2012.pdf. (Accessed 25 March 2012.)

Church, D. C. 1988. The Ruminant Animal, Digestive Physiology and Nutrition. Waveland Press.

Coghlin, C. L. 1944. Hydrogen sulphide poisoning in cattle. Can. J. Med. Vet. Sci. 8(4):111-113.

Cole, N. A., and R. W. Todd. 2008. Opportunities to enhance performance and efficiency through nutrient synchrony in concentrate-fed ruminants. J. Anin. Sci. 86:E318-E333.

Combs, G. F. 1998. The Vitamins. Fundamental aspects in nutrition and health. 2nd ed. Academic Press, London.

Conner, J. G., P. D. Eckersall, A. Wiseman, T. C. Aitchison, and T. A. Douglas. 1988. Bovine acute phase response following turpentine injection. Res. Vet. Sci. 44: 82-88.

Domby, E. M. 2011. The effect of feed additive program in steam-flaked corn diets containing wet distiller's grains on performance and carcass merit in yearling steers. Masters thesis. Colorado State Univ. Fort Collins.

Duckett, S. K. and J. G. Andrae, 2001. Implants strategies in an integrated beef production system. J. Anim. Sci. 85:823-840.

Duff, G. C., and M. L. Galyean. 2007. Board-invited review: Recent advances in management of highly stressed, newly received feedlot cattle. J. Anim. Sci. 85:823-840.

Ensley, S. M. 2011. Biofuels coproducts tolerance and toxicology for ruminants. Vet. Clin. North Am. Food Anim. Pract. 27:297-303.

Fakhruddin, P. D. Mathur, S. N. Sharma, and J. S. Yadav. 1987. Experimental studies on polioencephalomalacia (cerebrocortical necrosis) in goats induced by amprolium. Indian J. Anim. Sci. 57:377.

Gardner, B. A., H. G. Dolezal, L. K. Bryant, F. N. Owens, and R. A. Smith. 1999. Health of finishing steers: Effects of performance, carcass traits and meat tenderness. J. Anim. Sci. 77:3168-3175.

Goetsch, A. L., F. N. Owens. 1987. Effects of supplemental sulfate (Dynamate) and thiamin-HCl on passage of thiamin to the duodenum. Arch. Anim. Nutr. 12:1075-1083.

Gould, D. H., M. M. McAllister, J. C. Savage, and D. W. Hamar. 1991. High sulfide concentrations in rumen fluid associated with nutritionally induced polioencephalomalacia in calves. Am. J. Vet. Res. 52:1164-1169.

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

Gould, D. H. 2000. Update on sulfur-related polioencephalomalacia. Vet. Clin. North Am. Food Anim. Pract. 16:481-496.

Gould, D. H. 2008. Sulfur-related polioencephalomalacia in cattle. Univ of Neb. Storage of wet corn co-products Conf. May.

Grandin, T. 1997. Assessment of stress during handling and transport. J. Anim. Sci. 75:249-257.

Griffin, W. A., D. C. Adams, and R. N. Funston. 2009. The effect of delaying initial feedlot implant on body weight, average daily gain, and carcass characteristics of calf-fed steers. Prof. Anim. Sci. 25:145-149.

Handcock, D. L., J. F. Wagner, and D. B. Anderson. 1991. Effects of estrogens and androgens on animal growth. Pages 197-255 in growth regulation in farm animals: Advances in meat research. Volume 7. A.M. Pearson and T.R. Dutson, eds. Elsevier Appl. Sci., New York, NY.

Hutcheson, D. P. 1980. Observations on receiving new cattle. Texas Beef Conf., Amarillo, TX. April.

Hutcheson, D. P., and N. A. Cole. 1986. Management of transit –stress syndrome in cattle: nutritional and environmental effects. J. Anim. Sci. 62:555-560.

Jensen, R., L. A. Griner, and O. R. Adams. 1956. Polioencephalomalacia of cattle and sheep. J. Am. Vet. Med. Assoc. 129:311.

Johnson, R. W. 1997. Inhibition of growth by pro-inflammatory cytokines: An integrated review. J. Anim. Sci. 75:1244-1255.

Kandylis, K. 1983. Transfer of plasma sulfate from blood to rumen: a review. J. Dairy Sci. 66:2263-2270.

Klaassen, C. D. 1996. Casarett and Doull's Toxicology; The Basic Science of Poisons. Fifth Edition. McGraw-Hill Companies Inc.

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.

National Animal Health Monitoring System. 1999. http://www.aphis.usda.gov/animalhealth/ nahms /feedlot/downloads/feedlot99/Feedlot99_is_WaterQuality.pdf. Accessed September 30th 2012.

NRC. 1984. Nutrient Requirements of Beef Cattle. Sixth Revised Edition. National Academy Press. Washington, DC.

NRC. 2000. Nutrient Requirements of Beef Cattle. Seventh Revised Edition. National Academy Press. Washington, DC.

NRC. 2005. Mineral tolerance of animals. 7th Ed. Natl. Acad. Press, Washington, DC.

Merck Veterinary Manual. 1998. 8th Ed. Merck & Co.

Murray, R., V. Rodwell, D. Bender, K. M. Botham, P. A. Weil, and P. J. Kennelly. Harper's illustrated biochemistry. 2009. 28th Ed. The McGraw-Hill Companies, Inc.

Oliveira L. A. de, C. Jean-Blain, V. D. Corso, V. Benard, A. Durix, and S. Komisarczuk-Bony. 1996. Effect of a high sulfur diet on rumen microbial activity and rumen thiamine status in sheep receiving a semisynthetic, thiamine-free diet. Reprod. Nutr. Dev. 36:31.

Olkowski, A. A. 1997. Neurotoxicity and secondary metabolic problems associated with low to moderate levels of exposure to excess dietary sulfur in ruminants: a review. Vet. Hum. Toxicol. 39:355-360.

Rumsey, T. S., R. R. Oltjen, H. Swan, and D. J. Bolt. 1973. Physiological changes and blood lactic acid values in beef steers fed DES under ad libitum, restricted and compensatory conditions. J. Anim. Sci. 37:1201-1206.

Rumsey, T. S., H. F. Tyrrell, and P. W. Moe. 1980. Effect of diethylstilbestrol and synovex-s on fasting metabolism measurements of beef steers. J. Anim. Sci. 50:160-166.

Rumsey, T. S., H. F. Tyrrell, D. A. Dinius, P. W. Moe, and H. R. Cross. 1981. Effect of diethylstilbestrol on tissue gain and carcass merit of feedlot beef steers. J. Anim. Sci. 53:589-600.

Rumsey, T. S. 1982. Effect of synovex-s implants and kiln dust on tissue gain by feedlot beef steers. J. Anim. Sci. 54:1030-1039.

Rumsey, T. S, and A. C. Hammond. 1990. Effect of intake level on metabolic response to estrogenic growth promoters in beef steers. Anim. Sci. 68:4310-4318.

Rumsey, T. S, A. C. Hammond, and T. H. Elsasser. 1999. Responses to an estrogenic growth promoter in beef steers fed varying nutritional regimens. Anim. Sci. 77:2865-2872.

Samber, J. A., J. D. Tatum, M. I. Wray, W. T. Nichols, J. B. Morgan, and G. C. Smith. 1996. Implant program effects on performance and carcass quality of steer calves finishing for 212 days. J. Anim. Sci. 74:1470-1476.

Sarturi, J. O., G. E. Erickson, T. J. Klopfenstein, and C. D. Buckner. 2011. Ruminal degradable sulfur from organic and inorganic sources in beef cattle finishing diets. J. Anim. Sci. 89 (Suppl. 1):450.

Smith, L., H. Kruszyna, and R. P. Smith. 1977. The effect of methemoglobin on the inhibition of cytochrome c oxidase by cyanide, sulfide or azide. Biochem. Pharmacol. 26(23):2247-2250.

Sowell, B. F., J. G. P. Bowman, H. W. Sherwood, M. E. Branine, and M. E. Hubbert. 1998. Radio frequency technology to measure feeding behavior and health of feedlot steers. Appl. Anim. Behav. 59:275-282.

Spicer, E. M., and B. J. Horton. 1981. Biochemistry of natural and amprolium-induced polioencephalomalacia in sheep. Aust. Vet. J. 57:230-235.

Tillman, A.D., and J.R. Brethour. 1957. The effects of diethylstilbestrol upon gains, bone growth, bone composition, and calcium, phosphorus and nitrogen metabolism of lambs fed two levels of protein. J. Anim. Sci. 16:1033 (Abstract).

Underwood, E. J., and N. F. Suttle. 2001. The Mineral Nutrition of Livestock. 3rd ed. CABI Publishing, Cambridge.

CHAPTER II

SULFUR METABOLISM IN BEEF CATTLE

SUMMARY

Three experiments were conducted to evaluate sulfur metabolism in beef cattle. The first 2 experiments were conducted at Colorado State University's Agricultural Research Development and Education Center in Fort Collins, CO. The objective of the first experiment was to evaluate the effect of rumen fluid type on hydrogen sulfide (**H_2S**) production and pH *in vitro*. The objective of the second experiment was to evaluate the effect of rumen fluid type on sulfur (**S**) loss and dry matter disappearance (**DMD**) of common feedstuffs (**FS**). The final experiment was conducted at Southeast Colorado Research Center in Lamar, CO. The objective of this experiment was to evaluate the effect of feed additive program and variation in S intake on rumen fluid pH and rumen gas cap H_2S concentration.

In experiment 1, rumen fluid from 2 fistulated steers receiving a high roughage (**ROU**; 50% alfalfa hay, 50% corn silage) or a high concentrate-based diet (**CON**; 70% rolled corn, and 30% corn silage) was utilized to examine *in vitro* H_2S production of common beef cattle feedstuffs (**FS**). Rumen fluid was collected and combined in equal amounts from 2 rumen fistulated steers that had *ad libitum* access to ROU and mixed at a 3 to 1 ratio of artificial saliva to rumen fluid. Fermentation substrates included: corn, alfalfa, distiller's soluble (**DS**), dried distiller's grains (**DDG**), and wet distillers grain (**WDG**). Individual substrates (700mg) were added to separate 125 mL glass serum bottles (in triplicate) with 50 mL of rumen fluid-artificial saliva mixture. Fermentation bottles were capped with an air tight rubber stopper and incubated in a water bath for 24 h at 39°C. After 24 h of incubation, the total volume of gas produce was

measured and a 5 mL gas sample was obtained. Gas samples were then analyzed for H_2S concentration, after gas sampling, pH and DMD were determined. This experiment was then repeated using rumen fluid from steers fed CON. A FS by rumen fluid type (ROU vs. CON) interaction ($P < 0.001$) was detected for μmol of H_2S produced per mg of DMD. Distiller's soluble substrate produced less H_2S per mg of DMD when incubated with CON compared to ROU rumen fluid while corn, alfalfa, DDG and WDG produce greater μmol of H_2S per DMD when incubated in CON compared to ROU rumen fluid. Across diets (ROU vs. CON), fermentation of corn produced the lowest pH and fermentation of alfalfa produced the highest pH and across FS, ROU had a higher pH when compared to the CON rumen fluid. These data suggest that type of rumen fluid (ROU vs. CON) type and FS can influence the production of H_2S. Understanding factors that influence H_2S production within the rumen may be useful when formulating beef cattle diets.

In experiment 2, two rumen fistulated cross bred steers were fed a ROU for a minimum of 21 d prior to the initiation of this experiment. Samples were prepared for *in situ* digestibility by first being dried in a 60°C oven for 48 h and then ground through a 2 mm screen. *In situ* bags were incubated in 2 rumen fistulated steers for 0, 12, 24, or 36 h. Bags were dried in a 60°C forced air oven for 48 h, weighed, and DMD was calculated. Samples were then sent to a commercial laboratory for sulfur analysis. Samples from each FS that were washed but not fermented were also sent. Any bags that did not contain a minimal amount of the sample were composited with other bags of similar type and time to allow for sulfur analysis. A sample from the original FS was sent with each group of samples to be analyzed by the commercial laboratory, these data were averaged to obtain the number used for the original S concentration of the each FS. Steers were switched to a CON for 21 d and then *in situ* fermentation was

initiated again. Data were analyzed as a complete randomized design using PROC MIXED procedures of SAS. *In situ* bag was used as the experimental unit for all data analyzed.

The S concentration data for the FS were not statistically analyzed and were 0.12, 0.34, 0.70, and 0.77% (corn, alfalfa, WDG, and DDG, respectively). Over a 36 h period, DMD was greater ($P < 0.0001$) for FS incubated in the steers consuming the ROU when compared to steers consuming the CON. Corn had the greatest ($P < 0.0001$) percent of DMD in ROU and CON and alfalfa had the lowest percentage of DMD in ROU and CON ($P < 0.0001$; 94.71, 83.44, 77.32, and 58.70% ± 2.72; respectively). In CON, corn had the greatest percent S lost followed by alfalfa, then WDG and finally DDG (67.04, 61.86, 61.39, and 60.13% ± 12.527; respectively). However, in the ROU diet alfalfa had the great percent S lost followed by WDG, then corn and finally DDG (89.24, 74.23, 73.46, and 61.48% ± 12.527; respectively). A 4-way interaction ($P < 0.05$) was detected between diet, FS, steer, and time for percentage S loss. This interaction cannot readily be explained; however, this interaction suggests that the S loss from a FS, expressed as percentage of total S, is dependent upon the specific combination of diet, FS, steer, and time being examined and therefore may be of limited value to gauge potential PEM problems. No 3- or 4-way interactions were found when S loss per unit DMD was analyzed. When analyzing S loss per DMD the main effect of FS ($P < 0.0001$), DDG had the greatest amount of S released per DMD and corn released the least, in a high concentrate and high roughage diet (2.63 and 4.22 vs. 1.15 and 1.03 mg/mg ± 0.566) averaged over all time periods. These data suggest that there may be a difference in the release of S from different FS; this could be due to the ratio of S types in FS (i.e. organic to inorganic) or microbial population.

In experiment 3, Crossbred yearling steers (n = 432) were used to study the effects of Laidlomycin and Chlortetracycline (**LC**) vs. Monensin and Tylosin (**MT**) and variation in S

intake on rumen fluid pH and rumen gas H_2S concentration. An unbalanced randomized block design using a 2 × 2 factorial arrangement of treatments was utilized. Factors included feed additive (LC vs. MT) and S concentration (constant vs. variable). The variable concentration (**VAR**) was intended to simulate the use of random loads of WDG. Random numbers were generated for each d of the experiment. High S diets (S = 0.60% of DM) were fed to VAR on d associated with an even number. Low S diets (S = 0.48% of DM) were fed to CON all d of the experiment and to the VAR only on d associated with an odd number. From d 0 through 35, a high S meal supplement was fed to VAR on the appropriate d. Since variation in S concentration in WDG is driven by rate of inclusion and S concentration in DS, 2 DS based liquid supplements (low S, 0.99% vs. high S, 2.35%) were used to create the constant (CON) vs. VAR S intake from d 36 through slaughter. Sulfuric acid was added to the high S DS used to obtain the intended dietary S concentration. On d 35, 70, and 105 rumen fluid and gas cap samples were obtained via rumenocentesis from a subsample (3 hd/pen and 3 pens/treatment) of steers to determine rumen fluid pH and H_2S concentration. The effects of feed additive, dietary S, or the interaction on rumen fluid pH were not significant ($P > 0.38$). An interaction between feed additive and dietary S treatment ($P < 0.02$) existed suggesting that the effect of feed additive on H_2S concentration was influenced by dietary S. Steers fed the CON diet receiving MT exhibited lower H_2S concentration than steers fed LC (1053 vs. 2519 mg/L). Steers fed the VAR diet receiving MT exhibited a higher H_2S concentration than steers fed LC (2567 vs. 2187 mg/L). Rumen H_2S concentration was related to rumen fluid pH suggesting that management of rumen pH may be a key in dietary S management.

Key Words: Antibiotic, Hydrogen sulfide, *In vitro*, Ionophore, pH, Sulfur

INTRODUCTION

In a ruminant animal, sulfur (**S**) can enter the gastrointestinal tract via: 1) feed, 2) water, and/or 3) endogenous secretions (i.e. saliva). Sulfur can enter the animal as either organic forms, such as proteins or inorganic forms such as sulfates. Methionine, cysteine, homocysteine, taurine, cystathionine, cysteic acid, and cystine are all amino acids that contain S (NRC, 2005). The B vitamins, thiamin and biotin, also contain S. National Research Council (2000) describes the S requirement for feedlot cattle as 0.15% of diet dry matter, and the maximum tolerable limit is 0.4% of diet dry matter. Two classes of rumen microorganisms, assimilatory and dissimilatory, are responsible for S metabolism in the rumen (Gould, 2000; Underwood and Suttle, 2001). Underwood and Suttle (2001) suggested that assimilatory microorganisms only use organic forms of S. Other factors that can affect S metabolism in the rumen are fermentable carbohydrates, nitrogen, pH, and other minerals (Underwood and Suttle, 2001). Some sulfides are absorbed through the rumen wall and hydrogen sulfide (**H_2S**) is expelled through eructation. It is hypothesized that majority of the S flows out of the rumen into the omasum in forms of rumen undegraded protein, microbial protein, or inorganic forms of S. Little is known about S metabolism in the omasum. The majority of excess absorbed S is excreted in the urine as sulfate.

Sulfur content can vary greatly both within and between FS. The majority of S content of a typical finishing feedlot diet is contributed by the corn, even though corn only contains, on average, 0.11% S (NRC, 2000). This is due to the fact that corn makes up between 70 to 90% of a typical feedlot ration.

The growth of the ethanol industry has brought about higher corn prices and an abundance of distiller's grains and other ethanol co-products. Many producers have replaced a portion of the corn in feedlot diets with distiller's grains to try to mitigate high feed cost. The

ethanol process removes the starch from the grain, thereby concentrating the other nutrients (proteins, fats, and minerals) in distiller's grains and other by-products. Sulfuric acid is also used in the cleaning of ethanol plants and to manipulate the pH of the mash during fermentation (Ensley, 2011). This can end up in the distiller's grains and add to the S content of the by-product. For these 2 reasons the S content of distiller's grains greatly exceeds the original S concentration of the 0.11% of the corn used to make the ethanol. Therefore, replacing corn with distiller's grains in feedlot diets can cause the S intake of feedlot cattle to be elevated.

Total S concentration is the only measure currently used the develop diets to attempt to control S toxicity. Sarturi et al. (2011) suggests there are 2 types of S in a ruminant diet; rumen degradable S and rumen un-degradable S, thus suggesting the source and type of S should be considered when formulating diets. However, little is known about the ability of individual FS to contribute to the H_2S pool in the rumen or the rate of S release from these FS.

MATERIALS AND METHODS

Prior to the initiation of this experiment, care, handling, and sampling of animals as described herein were approved by the CSU Animal Care and Use Committee.

Experiment 1: The objective of this experiment was to determine the effect of FS on *in vitro* pH, DM disappearance, and H_2S production. Two crossbred rumen fistulated steers weighing approximately 900 kg and 6 years of age were used as rumen fluid donors. Approximately 3.8 L of rumen fluid was collected in the morning prior to being fed; on the d the fluid was to be used. Steers were housed in dirt surfaced pen measuring 40 x 6.1 m with a single automatic water fountain shared between every 2 pens. Feed was delivered to steers in fence-line (6.1 m in length) concrete feed bunks. Fluid was collected from 2 fistulated steers that had

ad libitum access to a high roughage-based diet (**ROU**; 50% Alfalfa hay, 50% corn silage) for 21 d prior to the initiation of the experiment. After restraining the steer, the fiber mat was manipulated to allow access to the liquid portion of the rumen and a plastic receptacle place in the fluid until full and then removed from the rumen. This was repeated until 1.9 L of fluid was collected. Rumen fluid was placed in a thermos that was pre warmed by filling it with warm tap water; the warm water was removed immediately prior to filling the thermos with rumen fluid. Upon arrival to the laboratory, rumen fluid was strained through 4 layers of cheesecloth. Equal volumes of rumen fluid from each steer were thoroughly mixed. A modified McDougall's (McDougall, 1948) buffer (9.8g $NaHCO_3$, 3.70g Na_2HPO_4, 0.57g KCl, 0.47g NaCl, 0.12g $MgSO_4*7H_2O$, 0.50g urea, and 0.04g $CaCl_2$) was then mixed with the fluid at a ratio of 3 to 1 buffer to rumen fluid. Feedstuffs (**FS**) evaluated; alfalfa hay, corn, distiller's solubles (**DS**), dried distiller's grains (**DDG**), and wet distiller's grains (**WDG**); were selected because of their common use in feedlot on the high plains and they represent the FS that contribute to the overall S load of a feedlot animal. Samples of these FS were collected from stock piles at Southeast Colorado Research Center (**SECRC**) or Agricultural Research, Development, and Education Center (**ARDEC**). Different sources of alfalfa hay were tested for S concentration and the source with the highest sulfur concentration was used. Samples of each commodity were placed in a bag and stored at -20°C until analyzed. The samples were dried in a 60°C oven for 48 h and ground through a 2 mm screen (Thomas Model 4 Wiley® mill, Swedesboro, NJ) prior to the initiation of the experiment, with the exception of DS which was unable to be ground due to its high viscosity. Seven hundred mg of each FS was added to separate 125 mL glass serum bottles. Then 50 mL of the rumen fluid buffer mixture was added to each bottle. All samples were run in triplicate. Three bottles per run were used as a negative control to adjust the dry matter

disappearance and endogenous H_2S production. Once the rumen fluid and buffer mix was added to a bottle, the bottle was then flushed with CO_2 and capped with a bytyl-rubber stopper. The bytyl-rubber stopper was held in place by crimping a metal sleeve over the top of the bytyl-rubber stopper. Once all the bottles were filled and capped, they were incubated in a water bath for 24 h at 39°C equipped with a continuous shaking apparatus.

After 24 h of incubation, the total volume of gas produced was measured by the displacement of water in an inverted 250 mL buret. After the total gas volume was measured and recorded, a 5 mL gas sample was obtained from each bottle and analyzed for H_2S concentration. A technique similar to that described by Kung et al. (1998) was used for the H_2S gas collection and quantification. Briefly, the gas sample was injected into 10 mL BD Vacutainer® (Franklin Lakes, NJ 07417) containing water (pH 8) followed by the addition of a diphenlamine HCl-ferric Cl reagent. After 25 minutes, duplicate samples of each 10 mL BD Vacutainer® were placed into a 96 well plate and read at 670 nm. Once gas samples were obtained from a 125 mL glass serum bottle, the rubber stopper was removed and a sample of approximately 1 mL of liquid was obtained and pH recorded using a field pH meter (eco Testr pH 2, EUTECH Instruments Oakton®, Vernon Hills, IL 60061). After all bottles were sampled the bottles were placed in a -20°C freezer for greater than 48 hours to inhibit fermentation. Later the bottles were placed in a force-air drying oven at temperatures greater than 60°C until the weight of the bottles stabilized and dry matter disappearance was calculated. All steps were repeated in a second run. Then the fistulated steers were acclimated to a high concentrate-based (**CON**; 70% cracked corn) diet and experiment was repeated using rumen fluid from steers on a CON.

Experiment 2: Samples of approximately 2 kg of WDG, DDG, corn, alfalfa, and DS were collected from stock piles at SECRC or ARDEC. Samples of each commodity were placed in a bag and stored in at -20°C until analysis.

Two crossbred rumen fistulated steers weighing approximately 900 kg and 6 years of age were used for this experiment. Steers were housed in dirt surfaced pen measuring 40 x 6.1 m with a single automatic water fountain shared between every 2 pens. Feed was delivered to steers in fence-line (6.1 m in length) concrete feed bunks.

Samples were prepared for *in situ* digestibility by first being dried in a 60°C oven for 48 h and the ground through a 2 mm screen (Thomas Model 4 Wiley® mill, Swedesboro, NJ). Additionally *in situ* digestibility was conducted using the standardized method described by Vanzant et al. (1998). Briefly, within each run triplicate polyester bags (2 cm x 5 cm; Ankom, Fairprot, NY) of 50 ± 20 mm mesh, with an approximate sample DM to surface area ratio of 10:1 mg/cm^2. Bags were pre-soaked in 39°C for 20 minutes, and then incubated in the rumens of 2 steers for 0, 12, 24, or 36 h. Bags were placed in the rumen in descending order by time and all bags were removed at the same time point. The polyester bags were suspended in the rumen in a large mesh bag which was originally placed under the fiber mat. The bags were immediately rinsed at removal as previously described (Vanzant et al., 1998). Bags were dried in a 60°C forced air oven for 48 h and then weighed and weights were recorded and dry matter disappearance was calculated. Blank bags were incubated to act as controls to adjust for washing errors. Samples were then sent to SDK Labs (Hutchinson, KS) for S analysis. Any bags that did not contain a minimal amount of the sample were composited with other bags of similar type and time to allow for S analysis. A sample from the original FS was sent with each group that was sent to SDK Labs, these data were averaged to obtain the initial S concentration of each FS.

Sulfur lost per gram of DMD was calculated by taking the original concentration of S in the FS multiplied by the grams of DM placed into the bag (grams of S in, **GSI**). Then the S concentration of the sample after incubation in the steer was multiplied by the gram of DM left in the bag (grams of S out, **GSO**). Then GSO was subtracted from GSI to calculate grams of S lost (**GSL**). Finally GSL was divided by DMD from that sample.

Experiment 3: Four hundred thirty two cross-bred yearling steers were selected from an initial group of 528 steers (mean weight 355 kg). Upon arrival, steers had access to long-stemmed grass hay and water. Steers were then trailed to Southeast Colorado Research Center Lamar, CO (**SECRC**), placed into pens, and fed a common diet until processing on December 9. Processing procedures included the application of lot tags and individual electronic identification tags, vaccination with Express 3 (Boehringer Ingelheim, St. Joseph, MO) respiratory vaccine, injection with Noromectin (Norboork Laboratories Limited, Newry, Co. Down, Northern Ireland), back pouring with Permectin CDS (KMG Bernuth Inc., Houston, TX) and drenching with Safe-Guard (Fenbendazole, Intervet/Schering-Plough Animal Health, Millsboro, DE) to control internal parasites, and implanting with Revalor-XS (40 mg Estradiol and 200 mg trenbolone acetate, Intervet/Schering-Plough Animal Health, Millsboro, DE) delayed-release implant. Steers that died during the course of the trial were necropsied to determine the cause of death and brains were sent to CSU diagnostic laboratory for signs of PEM.

Steers were weighed individually and assigned breed type scores on trial d -1 (December 9). Steers were then ranked by weight, and individuals that were beyond ± 2 SD from the mean were removed from the experiment. In addition, individuals showing health problems or excessive Brahman, Longhorn, or Dairy breeding were also excluded from the experiment. Remaining steers were assigned a random number from 1 to 1000 using the RAND function of

Microsoft® Excel 2003 (Microsoft Inc., Seattle, WA). A sufficient number of steers with the lowest random numbers were removed from further consideration for the experiment leaving 432 eligible steers. Steers were ranked by weight within breed type and divided into 8 weight block replicates. Within each breed type by weight block, each set of group of 6 ranked steers were assigned to treatments 1 – 6 using the lowest to highest random number assigned to the steers, respectively. This was repeated for each group of 6 ranked steers until all steers were assigned to treatment. Steers were returned through the processing chute on d 0 (December 10), individually weighed, and tagged with visual tags identifying experiment number (4), treatment (1 – 6), weight block replicate (1 – 8), and animal number within each pen (1 – 9). Steers were then sorted into treatment pens, and the experiment was started. Water equipment issues as described below prevented the application of 2 of the treatments. This changed the experiment design from a balanced design with 8 weight block replicates for each of 6 treatment combinations to an unbalanced design with 8 replicates for each of 2 treatments and 16 replicates for each of the 2 remaining treatments.

The experiment was originally designed as a balanced randomized block with a 2 x 3 factorial arrangement of treatments. However, due to water equipment problems the experiment was conducted as an unbalanced randomized block using a 2 x 2 factorial arrangement of treatments. Factors included were feed additive program (Monensin/Tylosin, Elanco Animal Health, Greenfield, IN versus Laidlomycin/Chlortetracycline, Alpharma Animal Health, Bridgewater, NJ) and dietary S treatment (constant versus variable). Specific dietary treatment combinations consisted of the following:

1. Constant S with Monensin/Tylosin (16 replicates);
2. Variable S with Monensin/Tylosin (8 replicates);

3. Constant S with Laidlomycin/Chlortetracycline (16 replicates); and
4. Variable S with Laidlomycin/Chlortetracycline (8 replicates).

The variable treatment was intended to simulate the use of random loads of WDG. Often the S concentration in these loads varies widely. The variation in S concentration in WDG is driven by the rate of inclusion and the S concentration in DS. The S concentration in DS is driven by the use of sulfuric acid to cleanse the production equipment. For the first 35 days of the experiment, sulfur flowers (100% elemental S) were added to the appropriate mineral supplement to create the high S diets on random days for the variable S treatments (Tables 2.2. and 2.3.). From d 36 through slaughter, 2 DS based liquid supplements were used to create the constant versus variable S intake treatments (Table 2.4.). Sufficient sulfuric acid was added to the high S DS used in the experiment to obtain the intended dietary S concentration for the variable treatment.

In mid-December the reverse osmosis (**RO**) treatments were abandoned due to a malfunction in the RO water system and all cattle received well water. In late March 2010, RO water was available and whenever possible all cattle received a mixture of RO and well water. Table 2.8. displays the average water sulfate concentration for all months for the trial. Water sulfate concentration averaged 1712 ± 131 mg/L throughout the experiment. Sulfate is approximately 33.4% elemental S; therefore, average S concentration in the water was approximately 572 ± 44 mg/L. If water consumption averaged 25 L per steer during the experiment, S intake from water was about 14.3 g per steers daily. To consider the added S from water as a percentage of dry matter intakes, 0.17% needs to be added to the diet S concentration.

Feed analysis results for the finishing diets are displayed by proposed sulfur concentration and feed additive treatment in Table 2.9. Analyzed results for most nutrients were

reasonably close to theoretical values for all treatment finishing diets. Analyzed diet dry matter and neutral detergent fiber concentrations were slightly lower than theoretical values. Analyzed diet CP, NPN, ether extract, calcium, and sulfur were slightly higher than analyzed values. The target sulfur concentration for the finishing diets was 0.34 and 0.50% as compared with 0.48 and approximately 0.60% for the analyzed sulfur concentration for the low and high sulfur diets, respectively. The theoretical difference in sulfur concentration between the low and high sulfur diets was targeted at 0.16%. The analyzed differential was approximately 0.12%. Other analyzed nutrient concentrations were similar between the high and low S concentration diets. Analyzed nutrient concentrations for the Monensin and Tylosin diets were similar to analyzed values for the Laidlomycin and Chlortetracycline diets.

Random numbers were generated for each day of the experiment using the RAND function of Excel. The high S diets were fed to the variable S intake treatments on days associated with an even random number. The low S diets were fed to the constant S treatments all days of the experiment and to the variable S treatments only on days associated with an odd random number. Table 2.5. shows the results of the randomization for the feeding schedule. The low S diets were fed 83 days while the high S diets were fed 76 days. There were 20 instances where the low S diets were fed for a single day and 25 instances where the high S diets were fed for a single day. On 13 and 10 occasions the low and high S diets were fed for 2 consecutive days, respectively. The low and high S diets were fed for 3 consecutive days during 7 and 4 instances, respectively. Only on 1 and 3 occasions, respectively, were the low and high S diets fed for 4 consecutive days. There were 3 instances where the random nature of the feeding schedule could not be maintained. On 1 occasion the delivery high sulfur DS to SECRC was delayed forcing the use of the low sulfur DS for 7 consecutive days. Likewise, on another

occasion, the delivery of the low sulfur DS to SECRC was delayed forcing the use of the high sulfur DS for 7 consecutive days. The last of the high sulfur DS was used on May 13 just 6 days prior to slaughter. Rather than go through the time and expense of manufacturing and delivering a small amount of high sulfur DS needed for only a couple of more days, the low sulfur DS was used for all treatments for the final 5 days of the experiment.

Diets were manufactured and fed 2 times per day starting with round 1 at 0730 h and starting with round 2 at approximately 1100 h. Feedbunks were evaluated each morning at 0630 h and each afternoon at 1600 h. Whenever bunks were observed empty for 2 consecutive mornings, the amount of feed delivered to each bunk was increased approximately 227 g DM per head. Conversely, if excess feed was observed in the bunk for 2 consecutive mornings, the amount of feed delivered to the bunk was decreased an appropriate amount to entice the steers to clean the bunk.

A starter and a series of step-up diets were used to acclimate the steers to steam-flaked corn (Table 2.6.). Diets were formulated to meet or exceed the requirements for all nutrients listed by NRC (2000). The starter diet was fed to all cattle prior to the initiation of treatments on d 0. Step-up 1 diets were fed starting with d 0 (December 10) through the round 1 feeding on d 6 (December 11, 6.5 days), step-up 2 diets were fed starting with the round 2 feeding on d 6 through the round 1 feeding on d 17 (December 22, 11 days), and step-up 3 diets were fed starting with the round 2 feeding on d 17 through the round 2 feeding on d 35 (January 14, 18 days). Finishing diets (Table 2.6.) were fed from d 36 (January 15) through the end of the experiment and were formulated to contain 2% crude protein equivalent from non-protein nitrogen, 4% neutral detergent fiber solely from corn silage as the roughage source in the diet, 1000 IU per 0.4536 kg DM vitamin A, and 15 IU 0.4536 kg DM vitamin E. Because of the

concentration of WDG in the finishing diets, CP concentration exceeded requirements listed by NRC (2000). Finishing diets for the Monensin/Tylosin treatments contained 30 g per 907 kg DM monensin and 10 g per 907 kg DM tylosin. Finishing diets for the Laidlomycin/Chlortetracycline treatments contained 11 g per 907 kg DM laidlomycin and 33.33 g per 907 kg DM chlortetracycline (target of 350 mg chlortetracycline per head daily). Vitamins, minerals, urea, and feed additives were added to each diet in the form of a meal supplement (Table 2.2., 2.3., and 2.7.).

Samples of feed ingredients and rations were obtained weekly. Dry matter of feed ingredients and rations were determined weekly at SECRC by drying a portion of each sample in a forced-air oven (60°C) for 48 h. Feed ingredient and ration samples were composited by month and sent to a commercial laboratory (SDK Labs, Hutchinson, KS) for routine nutrient analysis. Feed refusals were measured, and samples were obtained for DM analysis whenever feed became spoiled due to adverse weather and on weigh days. Feed refusal samples were dried at SECRC in a forced-air oven (60°C) for 48 h.

Dry matter deliveries for each treatment were calculated by multiplying the as-fed feed delivery recorded for each day by the average weekly dry matter concentration determined by drying oven for each diet. Dry matter refusals were calculated by multiplying the amount of feed weighed back for each pen by the dry matter concentration of each individual weigh-back as determined by drying oven. Dry matter intake for each pen was calculated by subtracting the amount of DM refused from total DM delivered and dividing the result by head-days for the pen.

Hydrogen sulfide concentration in the rumen gas cap was determined as described by Loneragan (1998). Rumen fluid samples were obtained as described by Garrett et al. (1999). Briefly a 4 inch 16 ga needle was inserted through the lower left flank, through the rumen wall,

and into the rumen of each steer to be sampled. Rumen fluid pH was then determined on a 1 to 2 ml sample of rumen fluid.

Statistical Analysis

Experiment 1: Data were analyzed as a randomized complete block design using PROC GLIMMIX of SAS (Statistical Analysis System, version 9.2, Cary, NC). Bottle, run, diet, and sample were included in the models as fixed variables. All variables were considered as classification variables. Bottle was used as the experimental unit for all data analyzed. Differences between treatment means were examined using the SLICEDDIFF option of the LSMEANS statement in SAS.

Experiment 2: Data were analyzed as a complete randomized design using PROC MIXED procedures of SAS (Statistical Analysis System, version 9.2, Cary, NC). Diet, steer, sample and time were included in the model as fixed variables. All variables were considered as classification variables. *In situ* bag was used as experimental unit for all data analyzed. Differences between treatment means were examined using the PDIFF option of the LSMEANS statement in SAS. Backwards selection regression methods were used to select the final model, highest order interactions were removed first. An interaction or variable was considered significant at $P < 0.05$.

Experiment 3: Data were analyzed as a randomized complete block design with repeated measures using PROC MIXED of SAS (Statistical Analysis System, version 9.2, Cary, NC). Feed additive treatment (**TRT**), sulfur treatment (**S**), TRT * S interaction, period (**PER**), PER * TRT, PER * S, and PER * TRT * S were included in the models as fixed variables. Weight block replicate (**REP**), TRT * REP, and S * REP were included in the model as a random

variables. All variables were considered as classification variables. The subject of the repeated statement was REP * TRT * S, autoregressive (AR1) covariance structure was used, and Kenward-Roger degrees of freedom were computed. Pen was used as the experimental unit for all data analyzed. Differences between treatment means were examined using the PDIFF option of the LSMEANS statement in SAS.

Increased variation at lower rumen pH suggested that a transformation of rumen gas cap concentration was warranted prior to statistical evaluation. After transforming the data using Log10 the variation observed at any given pH was more homogeneous and therefore better suited for regression analysis.

RESULTS AND DISCUSSION

Experiment 1: Figure 2.1. demonstrates the effect of FS and S concentration on total H_2S production. Presenting the data in this manner best demonstrates the impact of S concentration on H_2S production and pH. However, it must be noted that FS and S concentration are confounded because similar S concentrations were not represented cross all FS. There was no FS by diet interaction ($P < 0.05$) for the total H_2S production and H_2S production was not different between diets (high roughage versus high concentrate; $P > 0.05$); therefore, total H_2S production was averaged across diets. Figure 2.1. shows a linear relationship between S concentration and total H_2S production, as would be expected (i.e. as S concentration increases in FS total H_2S production also increases). This is consistent with May et al. (2010) who compared H_2S production *in vitro* of corn and sorghum distiller's grains at varying concentrations and reported an increase in H_2S production in as S concentration increased in the

substrate. Figures 2.2. and 2.3. demonstrate the effect of FS, S concentration, and diet type (ROU vs. CON) on μmol of H_2S per mg of DMD and rumen fluid pH, respectfully. A feedstuff by diet (ROU vs. CON) interaction ($P< 0.0001$) was detected for H_2S produced per unit of DMD. The interaction is due to DS producing less H_2S per mg of DMD in the CON when compared to the ROU while corn, alfalfa, DDG and WDG produce greater H_2S per DMD in the CON when compared to the ROU. A feedstuff by diet interaction ($P < 0.0001$) was also identified for pH (Figure 2.3.; non-parallelism of the lines). Across diets, fermentation of corn produced the lowest pH and fermentation of alfalfa produced the highest pH (6.16 and 6.64 ± 0.03, respectively). Furthermore, across FS the roughage diets had a higher pH when compared to the concentrate diet. These data suggest that as the S concentration increases in a FS, total H_2S production increases and as H_2S production increases pH also increases. This suggests that S is acting as a hydrogen sink to buffer rumen pH. Furthermore, corn produces less H_2S per DMD in the roughage diet when compared to a concentrate diet whereas DS produces more H_2S per DMD in a roughage diet when compared to a concentrate diet. These are the first data to demonstrate a differential production of H_2S per DMD for different FS. The reason for this change could be due to differences in microbial populations in the different rumen fluids, differences in S makeup in the FS (organic S verses inorganic S), or differences in the amount of readily fermentable starch across FS.

Experiment 2: Table 2.1. demonstrates the effect of diet on DMD and S disappearance of FS after 36 h of incubation in a rumen. The original S concentrations of the FS were not statistically analyzed 0.12, 0.34, 0.70, and 0.77% (corn, alfalfa, WDG, and DDG, respectively). These FS were chosen because they are commonly used in high plains feedlot diets and contribute greatly to the total S concentration of a feedlot diet. An attempt was made to evaluate

the disappearance of S from DS; however, due to small particle size, all of the DS washed out of each bag and no DS samples were available for analysis. The organic form of S in corn, WDG, and DDG should be very similar due to the fact the corn is the primary source of substrate for ethanol production; however, during the process of producing ethanol, sulfuric acid is used to maintain pH and it is used for cleaning of the distillation columns (Ensley, 2011; Uwituze et al., 2011). National Research Council (2000) reports that the S requirement of beef cattle is 0.15%, this means that the majority of a feedlot steers S requirement is supplied by corn (0.12%). Table 2.1. demonstrates that over a 36 h period DMD was greater ($P < 0.0001$) for FS in the steers consuming the ROU when compared to steers consuming the CON. This is consistent with other research (Johnson and McClure, 1972; Neuhold, 2009). Johnson and McClure (1972) and Neuhold (2009) found that ruminants consuming high roughage diets had greater cellulose digestion than ruminants consuming high grain diets. Corn had the greatest ($P < 0.0001$) DMD and alfalfa had the lowest DMD in ROU and CON ($P < 0.0001$; 94.71, 83.44, 77.32, and 58.70% ± 2.72%; respectively). The fiber content of these FS can help to explain this, NRC (2000) reports the percent fiber of these FS are 28.0, 6.9, and 2.3 (alfalfa, distiller's grains with soluble, and cracked corn; respectively). They also report the ADF content of these FS as 36.7, 21.3, and 3.3% (alfalfa, distiller's grains with soluble, and cracked corn; respectively). Due to the fiber content corn should have the greatest digestibility, alfalfa the lowest digestibility and distiller's grains an intermediate digestibility.

Table 2.1., also demonstrates the percent S lost at 36 h of fermentation in the rumen. In a high concentrate diet corn had the greatest percent of S lost followed by alfalfa, then WDG and finally DDG (67.04, 61.86, 61.39, and 60.13 ± 12.527%; respectively). However in the ROU alfalfa had the greatest percent S lost followed by WDG, then corn and finally DDG (89.24,

74.23, 73.46, and 61.48 ± 12.527%; respectively). Therefore, an interaction ($P < 0.004$) was found between diet consumed by the steer and type of FS. When analyzing percent S loss a 4-way interaction ($P < 0.05$) was detected between diet, FS, steer and time this interaction cannot be explained. This interaction cannot readily be explained; however, this interaction suggests that the S loss from a FS, expressed as percentage of total S, is dependent upon the specific combination of diet, FS, steer, and time being examined and therefore may be of limited value to gauge potential PEM problems. No 3- or 4-way interactions were found when S loss per unit DMD was analyzed. Limited research is available exploring the *in situ* release of S from FS in ruminants. Kahlon et al. (1975) evaluated different chemical forms of S (elemental, ammonium sulfate, L-methionine, sodium sulfate, sodium sulfide, calcium sulfate, and hydroxyl analog of methionine) *in vitro* and found a difference in the relative availability of the source of S to be converted into microbial protein, suggesting that there may be a difference in the "releaseablity" of S from different FS by rumen microbes depending on the chemical form of S in the FS.

Figures 2.4. and 2.5., demonstrate the percent loss of DM and S, respectively over time in a CON. Corn demonstrated the greatest amount of DMD at all time point; which would be consistent with the relative amount of readily digestible carbohydrates. The other FS have a slower rate of digestion due to the higher amounts of ADF. Figure 2.5., demonstrate the time by FS interaction ($P < 0.05$) that was found when analyzing S loss. This suggests that S source may influence the rate of S release in the rumen. Figures 2.6. and 2.7., demonstrate the percent loss of DM and S, respectively over time in a high roughage diet. From Figure 2.7., the disappearance of S appears more linear in a ROU then in a CON. This would suggest that S is released at a slower rate in a ROU when compared to a CON. Figures 2.5. and 2.7., also help to illustrate the diet by time interaction that is reported in Table 2.1.

The most appropriate way to evaluate the release of S from a FS maybe to evaluate the amount of S lost per unit DMD. Table 2.1., demonstrates the main effect of FS ($P < 0.0001$) with DDG having the greatest amount of S released per DMD and corn released the least, in a CON and ROU (2.63 and 4.22 vs. 1.15 and 1.03 ± 0.566) averaged over all time periods. This is consistent with the previous experiment which found that corn produced the lowest concentration of H_2S per unit of DMD; however, there was no attempt to determine the fate of the S in this experiment; therefore it could have resulted in microbial protein, H_2S, or by passed the rumen after being released. These data suggest that there may be a difference in the release of S from different FS in different diets; this could be due to the ratio of S types in FS (i.e. organic to inorganic) or due to the difference in microbial populations in a high pH environment verses a low pH environment. More research is needed to investigate the release of S in the rumen and the fate of that S once it is released from a FS.

Experiment 3: Figure 2.8. demonstrates the relationship between rumen fluid pH and H_2S concentration in the rumen gas cap. As pH is reduced average H_2S concentration is increased and the variation in H_2S at a given pH is also increased. Increased variation at lower rumen pH suggests that a transformation of either or both the rumen pH and rumen gas cap concentration is warranted prior to statistical evaluation. Figure 2.9. demonstrates a plot of H_2S data transposed using the Log_{10}. Variation observed at any given pH is more homogeneous and therefore better suited for regression analysis. Figures 2.8. and 2.9 agree with Experiment 1. Sulfur is acting as a hydrogen sink, thereby producing more H_2S to attempt to increase rumen pH.

Rumen gas cap H_2S concentration and rumen fluid pH are shown in Table 2.10. Rumen fluid pH data were analyzed as-is or as the inverse of pH. Regardless of which analysis was

used, the effects of feed additive program, dietary S treatment, or the interaction between feed additive and S treatment on rumen pH were not significant ($P > 0.38$). Transformation of the H_2S data affected the statistical analysis resulting in a significant ($P < 0.001$) interaction between feed additive and dietary S treatment suggesting that the effect of feed additive on H_2S concentration was influenced by dietary S treatment. There are a number of interpretations for these results. For the constant Monensin/Tylosin treatment, feeding diets with varying S concentration on random days resulted in a significant increase ($P < 0.001$) in H_2S concentration; however, for the Laidlomycin/Chlortetracycline treatment, varying S concentration only marginally affected ($P < 0.09$) H_2S concentration. An alternative interpretation suggests that for the constant dietary S treatment, the use of Laidlomycin/Chlortetracycline increased ($P < 0.0001$) H_2S concentration compared with Monensin/Tylosin while for the varying S treatment, choice of feed additive did not impact ($P > 0.19$) H_2S concentration. Quinn et al. (2009) conducted an experiment investigating the effect of 3 ionophores and 2 antibiotics on the production of H_2S *in vitro* with or without added S. Monensin, Tylosin, Laidlomycin, and Chlortetracycline were all investigated and found no impact on the *in vitro* the production of H_2S. The difference between these 2 experiments could be due to the type of experiment (*in vitro* vs. *in vivo*) or to the differences in S concentration Quinn et al. (2009) targeted 0.17 and 0.42% and in this experiment 0.48 and 0.60%, or finally it could be due to the source of S (elemental and sulfuric acid vs. sodium sulfate) used in the current experiment verses Quinn et al. (2009).

IMPLICATIONS

Excessive S intake has been linked to polioencephalomalacia in cattle which is a cause of needless pain and suffering for the animals and financial loss for producers. Currently the only

measurement used to balance rations and manage S intake is total dietary S. These data would suggest that total dietary S is a reasonably tool to use to manage H_2S production. However, more research is needed to investigate the effect of S source on S release and the fate of S in the rumen.

Sulfur appears to be acting as a hydrogen sink to mitigate rumen pH. However at low pH the S is not enough by itself to maintain ruminal pH. Also pH only explained 9 to 12% of the variation in H_2S production *in vitro* and *in vivo*. Therefore, factors such as S source (organic vs. inorganic), S release, microbial population, and eating habits need to be investigated more fully as ways to better control H_2S production *in vivo*, and reduce losses associated with S toxicity.

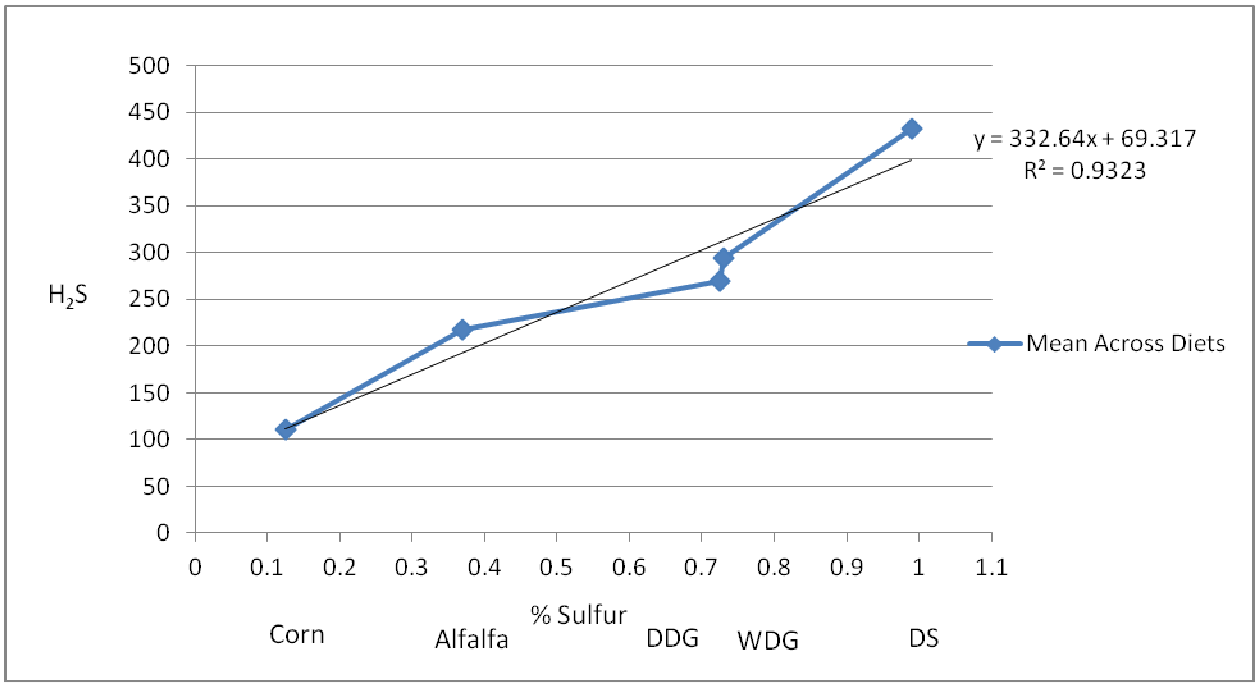

Figure 2.1. The influence of feedstuff and sulfur concentration on *in vitro* hydrogen sulfide production.

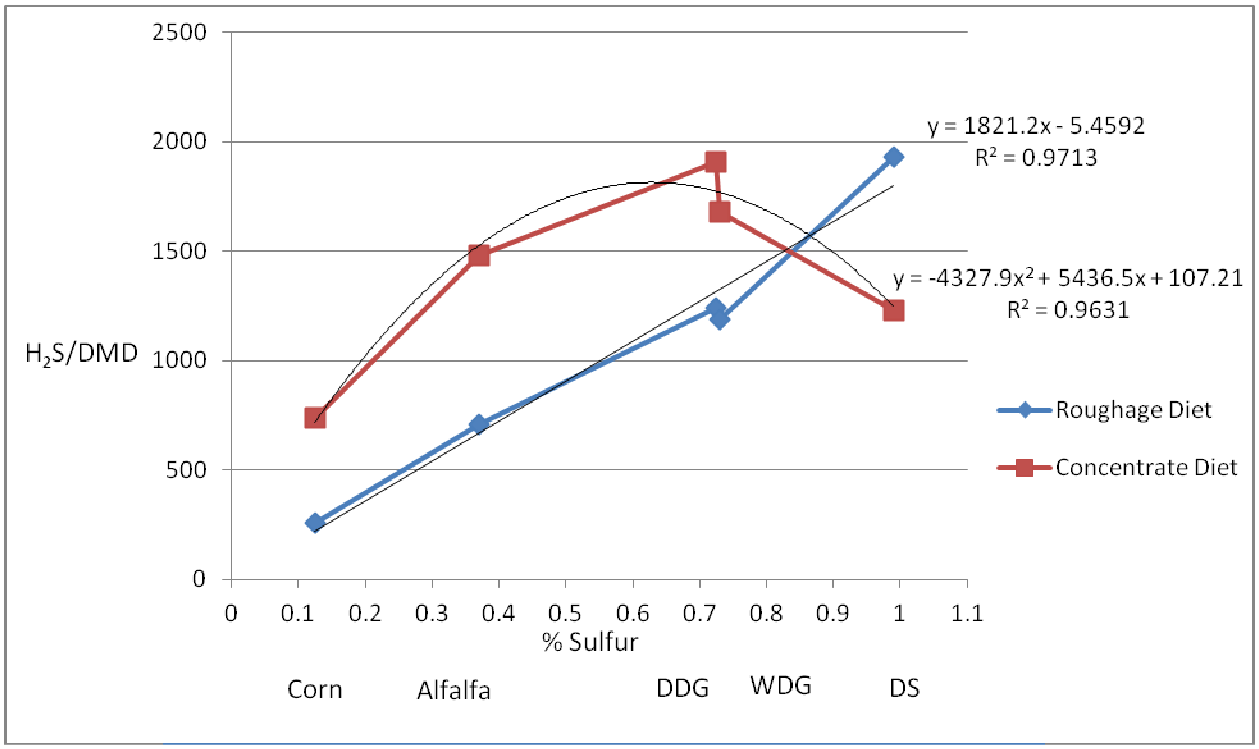

Figure 2. 2. The influence of feedstuff and sulfur concentration in the rumen of steers consuming high roughage diets or high concentrate diets on *in vitro* μmol of hydrogen sulfide production per gram of dry matter disappearance.

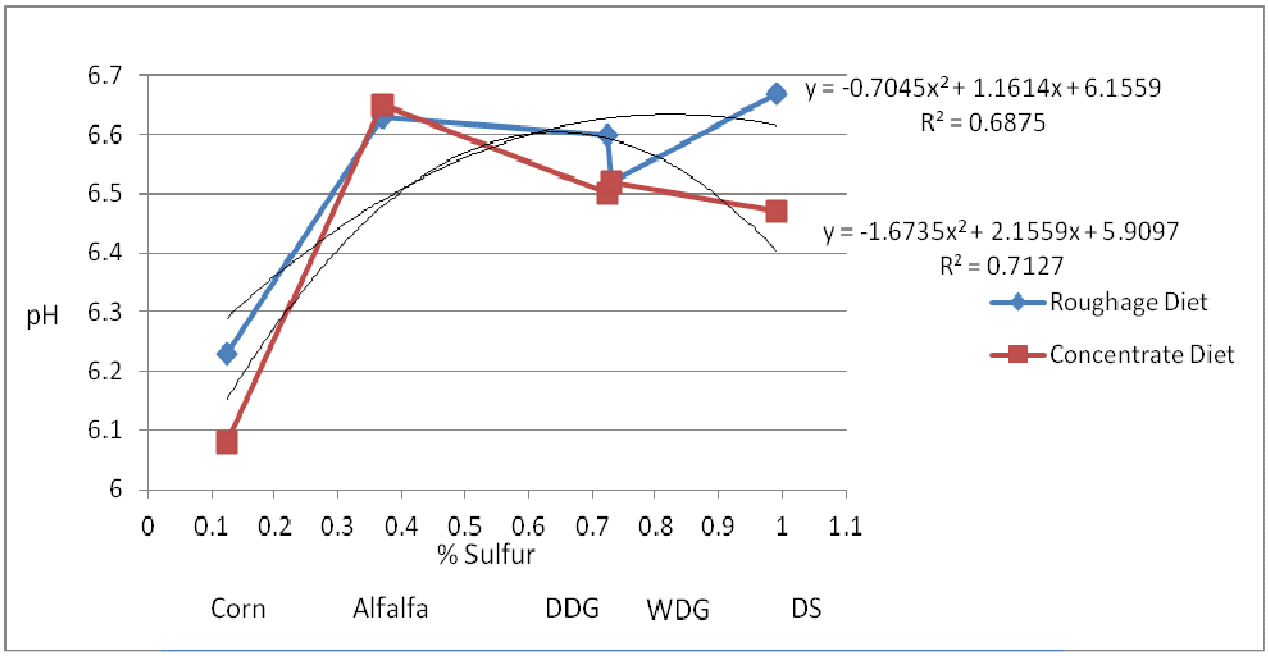

Figure 2.3. The influence of feedstuff and sulfur concentration in the rumen of steers consuming high roughage diets or high concentrate diets on *in vitro* pH.

Table 2.1. The effect of diet type on dry matter disappearance, sulfur disappearance, and sulfur loss per dry matter disappearance (mg/g) of common feeds stuffs used in feedlot diets.

	Concentrate Diets				Roughage Diets					*P* <		
Iteim[a]	Corn	Alfalfa	WDG	DDG	Corn	Alfalfa	WDG	DDG	SEM	Diet	FS	D*FS
Sulfur[a]	0.12	0.34	0.77	0.70	0.12	0.34	0.77	0.70				
%DMD[b]	83.44	58.70	70.38	64.25	94.71	77.32	84.10	79.26	2.720	0.0001	0.0001	0.0001
% SL[c]	67.04	61.86	61.39	60.13	73.46	89.24	74.23	61.48	12.527	0.0009	0.0015	0.0032
SL·DMD^{-1}[d]	1.15	1.33	1.57	2.63	1.03	1.73	3.59	4.22	0.566	0.007	0.0001	0.040

[a] FS = Feedstuff

[b] Percent sulfur in base sample, not analyzed.

[c] Percent dry matter lost in the 36 hour samples. A 4way interaction was detected Diet*Sample*Steer*Time.

[d] Percent sulfur lost in the 36 hour samples.

[e] Milligrams of sulfur lost per gram of dry matter lost averaged over the 3 time points (12, 24, and 36 hours).

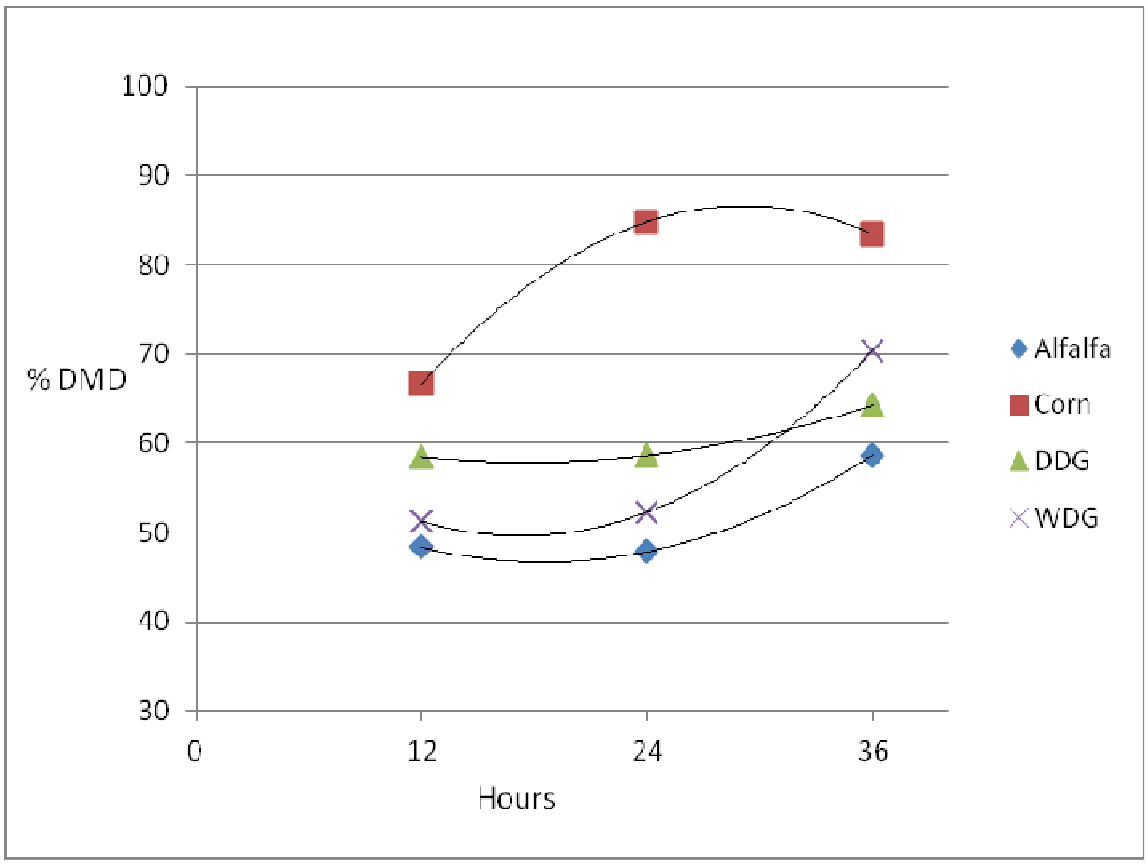

Figure 2.4. The effect of a high concentrate diet on the rate of dry matter disappearance (DMD) of common feedstuffs used in feedlot diets.

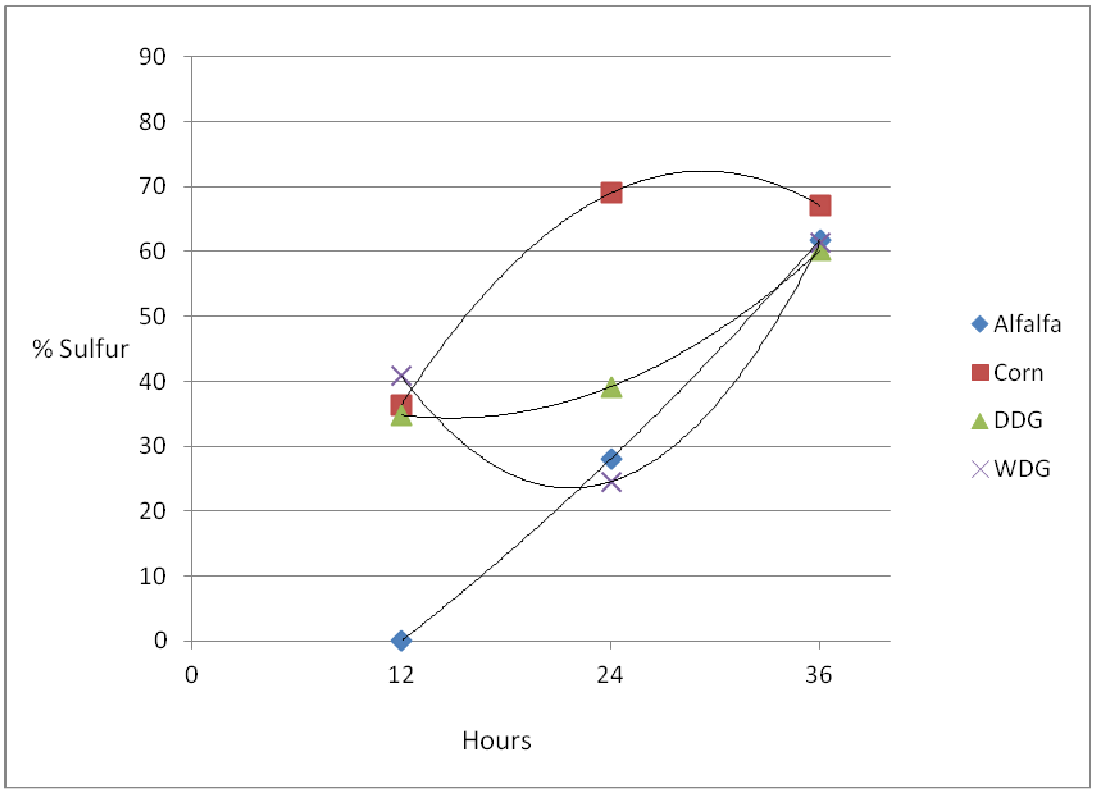

Figure 2.5. The effect of a high concentrate diet on the rate of sulfur loss of common feedstuffs used in feedlot diets.

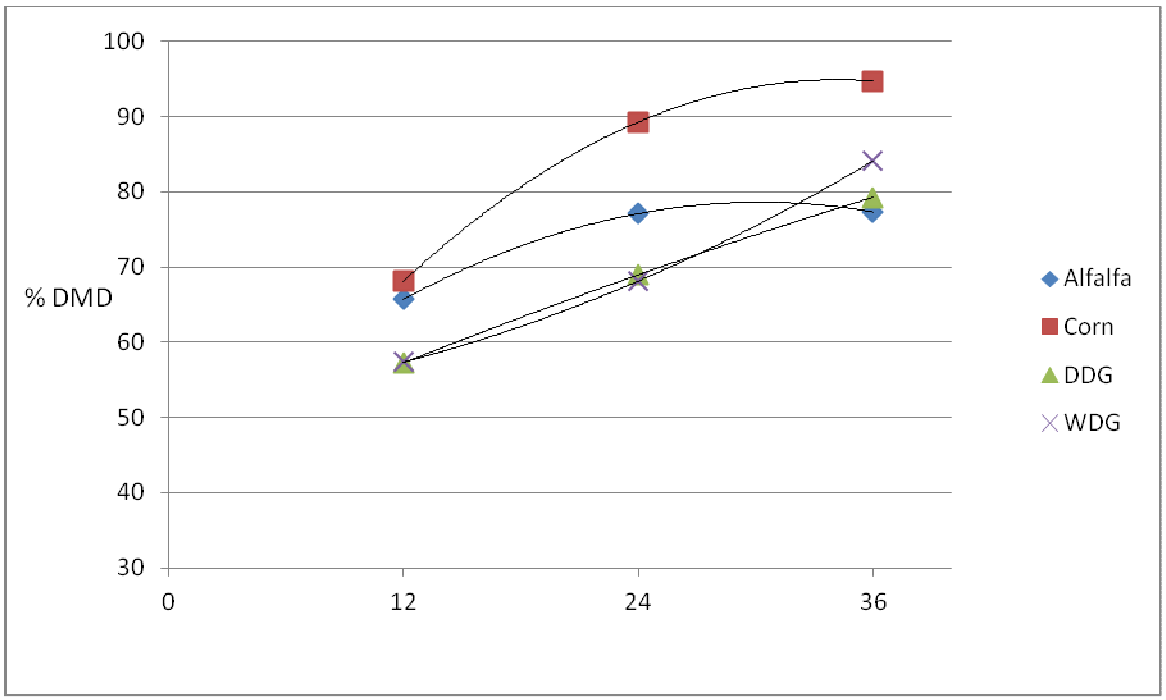

Figure 2.6. The effect of a high roughage diet on the rate of dry matter disappearance (DMD) of common feedstuffs used in feedlot diets.

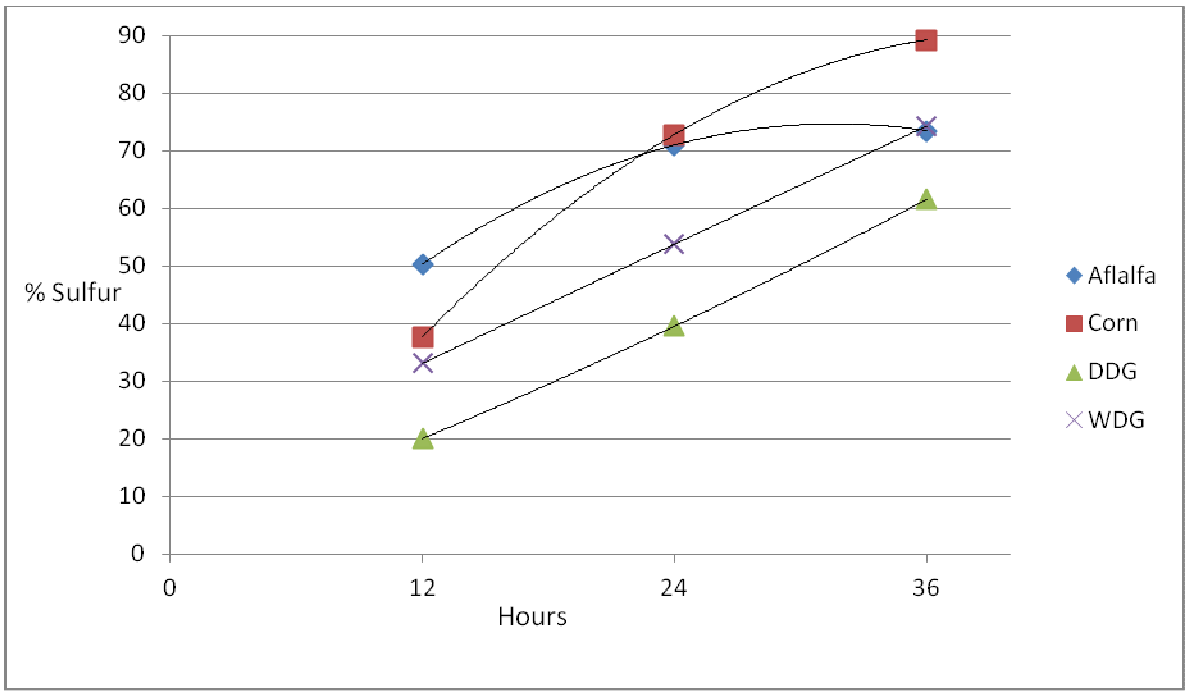

Figure 2.7. The effect of a high roughage diet on the rate of sulfur loss of common feedstuffs used in feedlot diets.

Table 2.2 As-fed composition of supplements used to establish low and high sulfur diets for treatments containing Monensin and Tylosin from d0 through d35 of the experiment.[1]

	Step-one Diets		Step-two Diets		Step-three Diets	
Ingredient[a]	Low S	High S	Low S	High S	Low S	High S
Urea	19.209	19.209	15.045	15.056	11.667	11.679
Limestone	47.914	47.914	52.587	52.555	49.706	49.756
Salt	13.782	13.782	10.795	10.803	8.372	8.380
Mineral oil	2.000	2.000	2.001	2.003	2.000	2.002
Min-Ad[b]			5.279	5.406	12.461	12.473
KCl[c]					3.998	4.002
Ground corn	10.864		9.223		7.697	
Sulfur flowers[d]		10.864		9.230		7.705
Monensin 80[e]	0.517	0.517	0.594	0.594	0.628	0.629
Tylosin 100[f]	0.275	0.275	0.216	0.216	0.168	0.067
TM premix[g]	4.410	4.410	3.455	3.457	2.679	2.682
Vit. A premix[h]	0.110	0.110	0.087	0.087	0.067	0.067
Vit. E premix[i]	0.919	0.919	0.719	0.594	0.558	0.559

[1]Adapted from Domby (2011).

[a] Percentage of as-fed.

[b] Min Ad Inc., Amarillo, TX. (21.45% calcium and 11.68% magnesium, DM basis).

c Potassium Chloride.

d Elemental sulfur, 100%.

e Monensin, 80 g per 0.4536 kg.

[f] Tylosin, 100 g per 0.4536 kg.

[g] Trace mineral premix: Cobalt, 500 mg/kg; Copper, 2.5%; Manganese, 6.25%; Zinc, 18.75%; Iodine, 630 mg/kg; and Selenium, 300 mg/kg.

[h] 50,000,000 IU vitamin A activity per 0.4536 kg.

[i] 90,000 IU vitamin E activity per 0.4536 kg.

Table 2.3. As-fed composition of supplements used to establish low and high sulfur diets for treatments containing Laidlomycin and Chlortetracycline from d0 through d35 of the experiment.[l]

	Step-one Diets		Step-two Diets		Step-three Diets	
Ingredient[a]	Low S	High S	Low S	High S	Low S	High S
Urea	18.978	18.978	14.949	14.941	11.641	11.641
Limestone	47.342	47.342	52.249	52.152	49.596	49.596
Salt	13.618	13.618	10.727	10.720	8.353	8.353
Mineral oil	2.000	2.000	2.001	2.000	2.000	2.000
Min-Ad[b]			5.252	5.373	12.436	12.436
KCl[c]					3.991	3.991
Ground corn	10.735		9.165		7.681	
Sulfur flowers[d]		10.735		9.160		7.681
Laidlomycin 50[e]	0.631	0.631	0.497	0.497	0.387	0.387
Chlortetracycline 90[f]	1.324	1.324	0.927	0.927	0.619	0.619
TM premix[g]	4.357	4.357	3.433	3.430	2.673	2.673
Vit. A premix[h]	0.108	0.108	0.086	0.086	0.067	0.067
Vit. E premix[i]	0.908	0.908	0.715	0.714	0.557	0.557

[l]Adapted from Domby (2011).
[a] Percentage of as-fed.
[b] Min Ad Inc., Amarillo, TX. (21.45% calcium and 11.68% magnesium, DM basis).
c Potassium Chloride.
d Elemental sulfur, 100%.
e Laidlomycin, 50 g per 0.4536 kg.
[f] Chlortetracycline, 90 g per 0.4536 kg .
[g] Trace mineral premix: Cobalt, 500 mg/kg; Copper, 2.5%; Manganese, 6.25%; Zinc, 18.75%; Iodine, 630 mg/kg; and Selenium, 300 mg/kg.
[h] 50,000,000 IU vitamin A activity per 0.4536 kg.
[i] 90,000 IU vitamin E activity per 0.4536 kg.

Table 2.4. As-fed ingredient composition and dry matter nutrient composition of the liquid supplements used to establish the low and high sulfur diets used from d36 through slaughter.[1]

Item	Low Sulfur	High Sulfur
Ingredient, % of as-fed		
Condensed corn distiller's soluble[a]	85.5500	83.5360
Crude glycerin[b]	12.5000	12.5000
Dry urea[c]	1.9500	1.9903
Sulfuric acid		1.9731
Nutrient[d]		
Dry Matter, % of as-fed[e]	42.96 ± 0.92	43.85 ± 0.41
Crude protein	25.42 ± 0.43	26.89 ± 0.57
Non-protein nitrogen[f]	14.21 ± 0.33	8.02 ± 1.83[g]
Neutral detergent fiber	3.62 ± 0.36	3.03 ± 0.32
Fat[h]	11.52 ± 0.26	11.92 ± 1.11
Calcium	0.27 ± 0.02	0.21 ± 0.01
Phosphorus	1.29 ± 0.03	1.28 ± 0.03
Potassium	1.98 ± 0.03	2.02 ± 0.05
Magnesium	0.61 ± 0.01	0.56 ± 0.01
Sulfur	0.99 ± 0.02	2.35 ± 0.06

[1] Adapted from Domby (2011).
[a] Quality Distiller's Grains, Hereford, TX.
[b] Added to improve flow rate during winter.
[c] Needed in the high sulfur liquid to help maintain pH above 2 facilitating transport of the product.
[d] Percentage of DM ± standard error of the mean unless stated otherwise.
[e] As-received moisture determined by Karl-Fischer methodology. DM = 100 – moisture.
[f] Crude protein equivalent.
[g] Non-protein nitrogen averaged 16.25 ± 0.14 for January and February and only averaged 2.53 ± 0.64 for March, April, and May.
[h] Fat was determined by acid hydrolysis.

Table 2.5. Randomized feeding schedule results for the low and high sulfur diets.[1]

Consecutive Days Fed	Low Sulfur Diet Episodes	Total Days	High Sulfur Diet Episodes	Total Days
1	20	20	25	25
2	13	26	10	20
3	7	21	4	12
4	1	4	3	12
5	1	5	0	0
6	0	0	0	0
7	1	7	1	7
Sum		83		76

[1]Adapted from Domby (2011).

Table 2.6. Ingredient and theoretical nutrient concentration for the starter, step-up, and finishing diets used for the feed additive and dietary sulfur experiment.[1]

Item[a]	Starter	Step-one	Step-two	Step-three	Finish
Ingredient					
Corn silage	36.917	20.716	15.066	9.416	9.978
Steam-flaked corn	28.173	27.497	42.648	57.610	45.598
Alfalfa hay	20.000	20.000	10.000		
DDG[b]	10.646				
WDG[c]		30.000	30.000	30.000	30.000
Yellow grease/tallow[d]					0.383
Corn steep liquor	3.000				
Liquid supplement[e]					11.839
Supplement[f]	1.264	1.787	2.286	2.974	2.202
Nutrient					
Dry matter, % of as-fed	50.714	47.225	49.821	52.738	49.241
Crude protein	14.000	18.073	17.204	16.298	17.422
Non-protein nitrogen	1.000	1.000	1.000	1.000	2.000
Acid detergent fiber	19.682	19.349	14.868	10.377	10.524
Neutral detergent fiber	30.412	31.380	25.745	20.075	20.086
Effective NDF	19.706	15.643	9.980	4.313	4.256
Crude fiber	16.862	14.627	10.837	7.039	7.069
Forage NDF[h]	24.000	18.000	11.000	4.000	4.000
NEm, Mcal/kg DM	1.812	1.743	1.868	1.983	2.035
NEg, Mcal/kg DM	1.157	1.144	1.258	1.367	1.409
Ether extract	4.650	6.142	6.562	6.967	7.500
Calcium	0.700	0.700	0.700	0.700	0.700
Phosphorus	0.310	0.421	0.431	0.440	0.553
Potassium	1.146	0.994	0.818	0.700	0.866
Magnesium	0.250	0.261	0.250	0.250	0.257
Sulfur[g]	0.216	0.31/0.50	0.29/0.50	0.30/0.50	0.34/0.50
Vitamin A, IU/kg DM	22.050	22.050	22.050	22.050	22.050
Vitamin E, IU/kg DM	0.331	0.331	0.331	0.331	0.331

[1] Adapted from Domby (2011).
[a] Percentage of DM unless stated otherwise.
[b] Dried distiller's grains.
[c] Wet distiller's grains.
[d] Yellow grease fed through March, 2010. Tallow fed during April and May, 2010.
[e] Refer to Table 4 for the ingredient and analyzed nutrient concentration for the liquid supplement.
[f] Refer to Tables 2 and 3 for the ingredient composition of the step-one, step-two, and step-three supplements and Table y for the starter and finishing diet supplements.
[g] First number in a column refers to the constant S treatments. The second number refers to the high S diets.

Table 2.7. As-fed ingredient composition of the starter and finishing diet supplements used for the feed additive and sulfur experiment.[1]

Ingredient[a]	Starter Diet	Monensin/Tylosin Finish Diet	Laidlomycin/Chlortetracycline Finish Diet
Urea	21.683	2.273	2.272
Limestone	47.927	78.555	78.556
Salt	17.979	11.334	11.334
Mineral oil	1.999	2.001	2.001
Min-Ad[b]	3.529		
Ground corn		0.288	
Monensin 80[c]		0.850	
Tylosin 100[d]		0.226	
Laidlomycin 50[e]			0.525
Chlortetracycline 90[f]			0.840
TM premix[g]	5.539	3.627	3.627
Vit. A premix[h]	0.144	0.090	0.090
Vit. E premix[i]	1.199	0.756	0.756

[1]Adapted from Domby (2011).
a Percentage of as-fed.
[b] Min Ad Inc., Amarillo, TX. (21.45% calcium and 11.68% magnesium, DM basis).
c Monensin, 80 g per 0.4536 kg. Finish diet contained 30 g per 907 kg of dry matter.
[d] Tylosin, 100 g per 0.4536 kg. Finish diet contained 10 g per 907 kg of dry matter.
e Laidlomycin, 50 g per 0.4536 kg. Finish diet contained 11 g per 907 kg of dry matter.
[f] Chlortetracycline, 90 g per 0.4536 kg. Finish diet contained 33.33 g per 907 kg dry matter to provide for 350 mg per head daily.
[g] Trace mineral premix: Cobalt, 500 mg/kg; Copper, 2.5%; Manganese, 6.25%; Zinc, 18.75%; iodine, 630 mg/kg; and Selenium, 300 mg/kg.
[h] 50,000,000 IU vitamin A activity per 0.4536 kg.
[i] 90,000 IU vitamin E activity per 0.4536 kg.

Table 2.8. Sulfate concentration (mg/L) in water consumed during the feed additive and dietary sulfur experiment.[1]

Date of sample	400 Alley	600 Alley	Average
January 6, 2010	1690	1660	1675
January 13, 2010	1340	1300	1320
January 20, 2010	1190	1310	1250
January 27, 2010	1660	1810	1735
February 3, 2010	2050	1870	1960
February 10, 2010	1900	2130	2015
February 17, 2010	2050	1940	1995
February 24, 2010	1840	1850	1845
March 3, 2010	2325	2475	2400
March 10, 2010	1875	1975	1925
March 17, 2010	1775	2125	1950
March 24, 2010	1925	788	1357
March 31, 2010	6	2350	1178
April 7, 2010	2300	2100	2200
April 14, 2010	2525	2350	2438
April 21, 2010	60	25	42
April 28, 2010	2350	2525	2438
May 5, 2010	1400	1400	1400
May 12, 2010	1430	1400	1415
Average	1668	1757	1712
Standard error	156	142	131

[1]Adapted from Domby (2011).

Table 2.9. Dry matter nutrient concentration in finishing diets as determined by laboratory analysis.[1]

	Low Sulfur		High Sulfur	
Item[a]	R/T	C/A	R/T	C/A
Dry matter[b]	46.99 ± 0.39	46.90 ± 0.25	48.08 ± 0.59	48.44 ± 0.60
Crude protein	18.74 ± 0.28	18.48 ± 0.18	18.41 ± 0.34	18.08 ± 0.50
Non-protein nitrogen	2.33 ± 0.06	2.36 ± 0.05	2.29 ± 0.09	2.20 ± 0.14
Neutral detergent fiber	18.04 ± 0.25	17.84 ± 0.27	17.79 ± 0.24	18.53 ± 0.87
Ether extract	8.65 ± 0.18	8.58 ± 0.15	8.23 ± 0.37	7.82 ± 0.42
Calcium	0.91 ± 0.05	0.90 ± 0.03	0.83 ± 0.03	0.89 ± 0.05
Phosphorus	0.57 ± 0.008	0.57 ± 0.005	0.53 ± 0.02	0.49 ± 0.03
Potassium	0.89 ± 0.01	0.88 ± 0.009	0.86 ± 0.01	0.86 ± 0.03
Magnesium	0.28 ± 0.004	0.27 ± 0.002	0.27 ± 0.004	0.26 ± 0.007
Sulfur	0.48 ± 0.007	0.48 ± 0.005	0.62 ± 0.02	0.58 ± 0.03

[1]Adapted from Domby (2011).
[a] Raw mean ± standard error of the mean. Dry matter basis unless stated otherwise.
[b] As-fed basis.

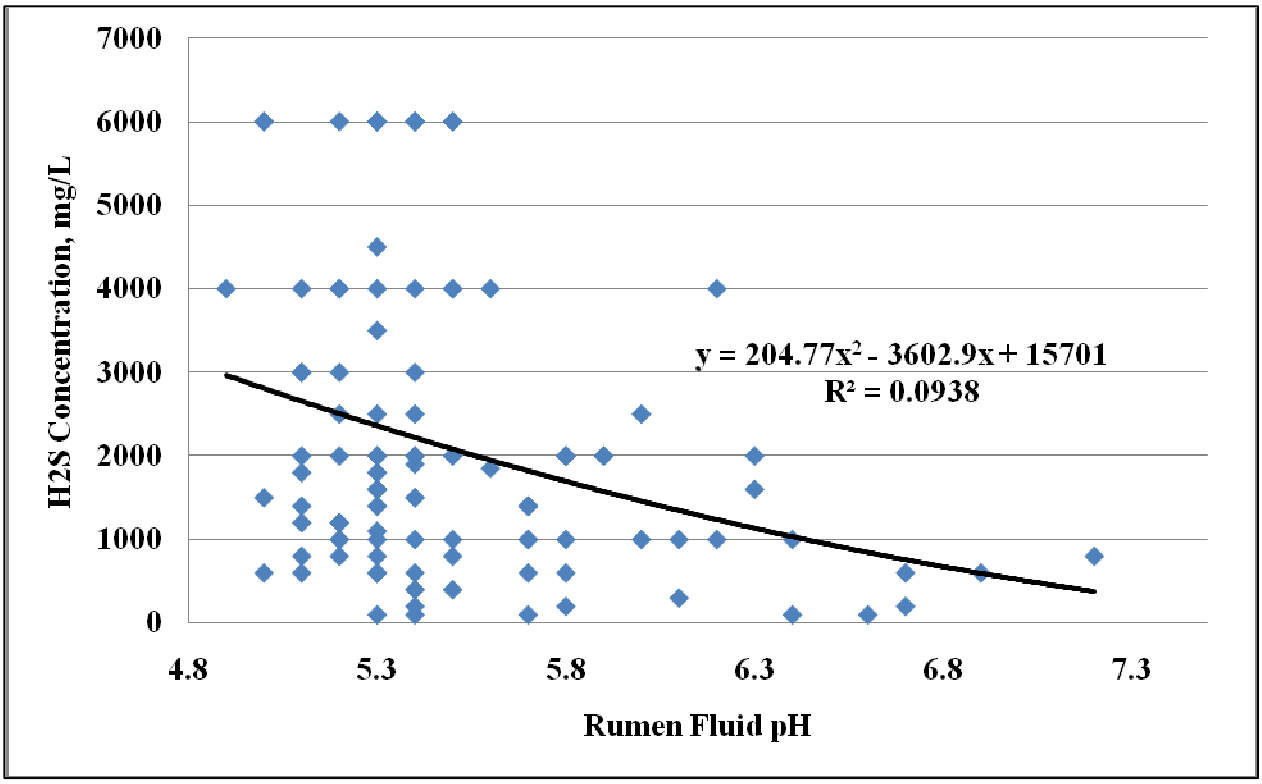

Figure 2.8. Effect of rumen fluid pH on hydrogen sulfide concentration in the rumen gas cap.

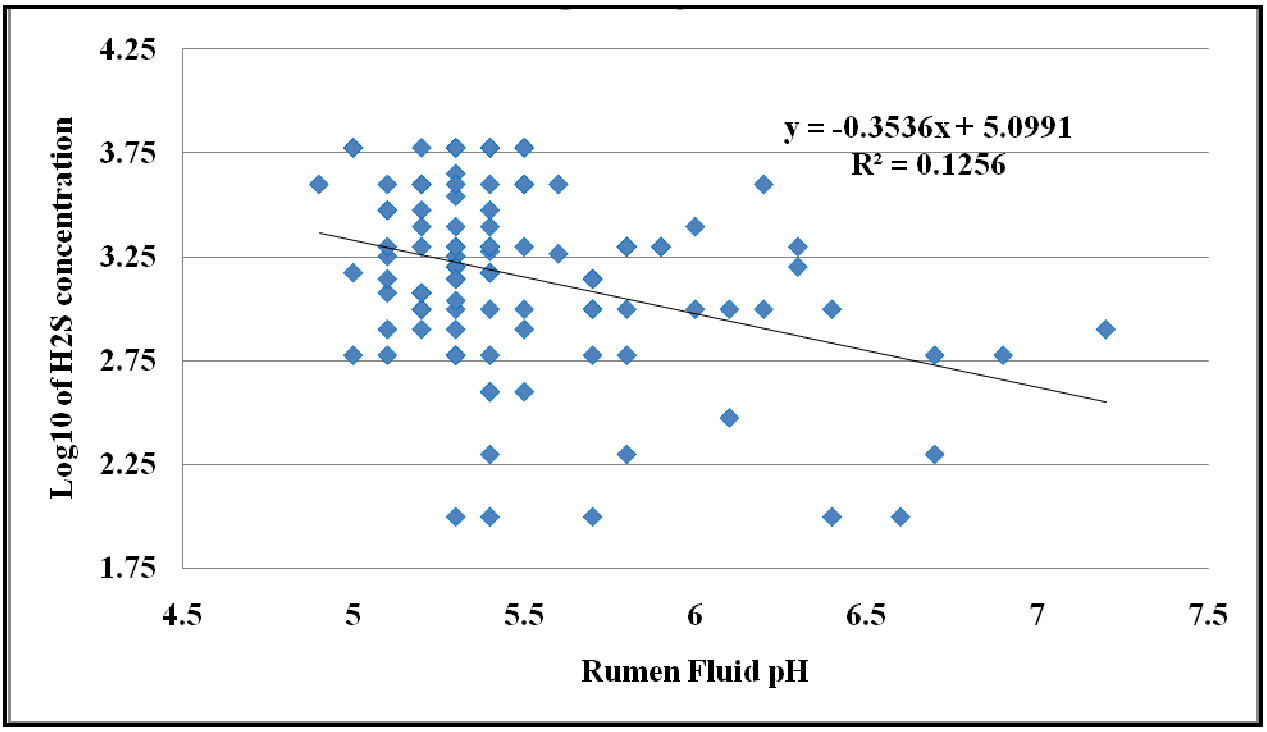

Figure 2.9. Effect of rumen fluid ph on the Log10 of hydrogen sulfide concentration in the rumen gas cap.

Table 2.10. The effect of feed additive and dietary sulfur treatment on rumen fluid pH and hydrogen sulfide concentration (mg/L) in the rumen gas cap.

	Monensin/Tylosin		Laidlomycin/Chlorte tracycline			Prob. > F		
Item	CON[a]	VAR[b]	CON	VAR	SEM[c]	Additive	Sulfur	A x S[d]
pH	5.56	5.53	5.43	5.56	0.092	0.5954	0.5751	0.3847
1/pH[e]	5.53	5.50	5.41	5.52	0.083	0.5591	0.6747	0.4257
H_2S	1053	2567	2519	2187	353	0.1324	0.1024	0.0127
Log_{10} H_2S[f]	697	1881	2089	1366	284	0.0296	0.1077	0.0002

[a] Constant diet sulfur concentration.
[b] Variable diet sulfur concentration.
[c] Standard error of the mean.
[d] Feed additive program by sulfur treatment interaction.
[e] Data analyzed as the inverse of pH. Least squares means for the inverse of pH transposed back to pH. Standard error of the mean is an approximation calculated from the inverse of pH least squares means ± the standard error for the inverse of pH.
[f] Data analyzed as the Log_{10} of the H_2S concentration. The antilog of the Log_{10} least squares means was calculated to transpose data back to mg/L. Standard error of the mean is an approximation calculated from determining the antilog of Log_{10} least square means ± the standard error for the Log_{10} least squares means.

LITERATURE CITED

Domby, E. M. 2011. The effect of feed additive program in steam-flaked corn diets containing wet distiller's grains on performance and carcass merit in yearling steers. Master thesis. Colorado State Univ. Fort Collins.

Ensley, S. M. 2011. Biofuels coproducts tolerance and toxicology for ruminants. Vet. Clin. North Am. Food Anim. Pract. 27:297-303.

Garrett, E. F., M. N. Pereira, K. V. Nordlund, L. E. Armentano, W. J. Goodger, and G. R. Oetzel. 1999. Diagnostic methods for the detection of subacute ruminal acidosis in dairy cows. J. Dairy Sci. 82:1170-1178.

Gould, D. H. 2000. Update on sulfur-related polioencephalomalacia. Vet. Clin. North Am. Food Anim. Pract. 16:481-496.

Johnson, R. R., and K. E. McClure. 1972. High fat rations for ruminants. I. The addition of saturated and unsaturated fats high roughage and high concentrate rations. J. Anim. Sci. 34:501-509.

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

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

Loneragan, G. H., D. H. Gould, R. J. Callan, C. J. Sigurdson, and D. W. Hamar. 1998. Association of excess sulfur intake and an increase in hydrogen sulfide concentrations in the ruminal gas cap of recently weaned beef calves with polioencephalomalacia. J. Am. Vet. Med. Assoc. 213:1599-1604.

May, M. L., J. C. DeClerck, J Leibovich, M. J. Quinn, N. DiLorenzo, D. R. Smith, K. E. Hales, and M. L. Galyean. 2010. Corn or sorghum wet distillers grains with soluble in combination with steam-flaked corn: In vitro fermentation and hydrogen sulfide production. J. Anim. Sci. 88:2425-2432.

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

Neuhold, K. L. 2009. An evaluation of direct-fed microbial production for yearling steers in wet distiller's grain diets and the effect of ensilage storage duration and proportion on *in situ* dry matter disappearance. Master thesis. Colorado State Univ. Fort Collins.

NRC. 2000. Nutrient Requirements of Beef Cattle. Seventh Revised Edition. National Academy Press. Washington, DC.

NRC. 2005. Mineral tolerance of animals. 7th Ed. Natl. Acad. Press, Washington, DC.

Quinn, M. J., M. L. May, K. E. Hales, N. DiLorenzo, J. Leibovich, D. R. Smith, and M. L. Galyean. 2009. Effect 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 diet substrate with or without added sulfur. J. Anim. Sci. 87:1705-1713.

Sarturi, J. O., G. E. Erickson, T. J. Klopfenstein, and C. D. Buckner. 2011. Ruminal degradable sulfur from organic and inorganic sources in beef cattle finishing diets. J. Anim. Sci. 89 (Suppl. 1):450.

Statistical Analysis System. 2003. The SAS System for Windows. Release 9.3.1. SAS Institute Inc. Cary, NC.

Underwood, E. J., and N. F. Suttle. 2001. The Mineral Nutrition of Livestock. 3rd ed. CABI Publishing, Cambridge.

Uwituze, S., G. L. Parsons, K. K. Karges, M. L. Gibson, L. C. Hollis, J. J. Higgins, and J. S. Drouillard. 2011. Effects of distillers grains with high sulfur concentration on ruminal fermentation and digestibility of finishing diets. J. Anim. Sci. 89:2817-2828.

Vanzant, E. S., R. C. Cochran, and E. C. Titgemeyer. 1998. Standardization of *in situ* techniques for ruminant feedstuff evaluation. J. Anim. Sci. 76:2717-29

CHAPTER III

MANAGEMENT STRATEGIES TO IMPROVE PERFORMANCE AND HEALTH IN NEWLY WEANED BEEF CATTLE

SUMMARY

Two experiments were conducted to evaluate receiving strategies to improve feedlot performance and cattle health in newly weaned beef cattle. The first experiment was conducted at Eastern Colorado Research Center in Akron, CO (**ECRC**). The objective of this experiment was to evaluate the timing of growth promotant implant either upon arrival to the feedlot or delayed until d 28. The second experiment was conducted at Colorado State University's Agricultural Research, Development, and Education Center in Fort Collins, CO (**ARDEC**). The objective of this experiment was to evaluate the effect of feeding strategy on cattle performance, morbidity, and mortality 30 d immediately post-weaning.

In experiment 1, 442 newly weaned Angus and Angus crossbred steers (initial BW = 234 ± 40.4 kg) were selected from an initial group of 453 from 3 ranches in CO. These steers were used to evaluate the effect of implant timing on feedlot performance and health. This experiment was conducted as a randomized complete block design. Appropriate response variables were analyzed on both and individual steer and on a pen basis. Treatments including an implant (Revalor®-XS) at arrival on d 0 of the experiment (**ARR**) versus the same implant administered 28 d later, delayed (**DEL**). Both treatments received a common diet and diet changes were made on the same d for both treatments. Individual live BW was recorded on d 0, 28, 56, 112, 169, and prior to harvest. On d 0, 5 steers from each pen were vaccinated with 2 mL of ovalbumin (**OVA**) subcutaneously and 1 mL was administered intradermally. On d 28 the same 5 steers

received a booster of the OVA and 5 novel steers also receive a vaccination of OVA. This allowed d 0 and d 28 primary responses to be evaluated along with a d 28 secondary immune response.

On an individual steer basis ARR steers had a tendency ($P < 0.07$) for heavier BW then DEL steers on d 28(293 vs. 291 ± 1.7 kg, respectively). Likewise ADG from d 0 to d 28 favored the ARR treatment over the DEL (1.80 vs. 1.71 ± 0.056 kg, respectively). However, by d 56 BW were again similar ($P > 0.11$). Final BW were different when comparing treatments ($P < 0.05$) at harvest time; DEL treatment weighed more than ARR (636 vs. 627 ± 8.3 kg, respectively). This increased final BW is consistent with the overall ADG where the DEL treatment out gained ARR (1.78 vs. 1.74 kg ± 0.029, respectively). Steers in the ARR treatment had increase DMI over the DEL treatment from d 0 to d 112 ($P < 0.018$). However, in from day 113 through harvest there was no difference ($P > 0.20$) between treatments. Therefore, total DMI was greatest ($P < 0.06$) for the ARR treatment when compared to the DEL (9.81 vs. 9.56 ± 0.194 kg, respectively). Arrival steers had a lower gain-to-feed (0.175 vs. 0.186 ± 0.0033) and a higher feed-to-gain ratio (5.72 vs. 5.40 ± 0.101) when compared to DEL steers ($P < 0.0001$). Hot carcass weight was different ($P < 0.0001$) with the DEL have approximately 6 kg heavier weight than ARR (384 vs. 378 ± 4.7 kg, respectively). Carcass quality grade, yield grade, dressing percentage, and longissimus dorsi area were not different between treatments ($P > 0.40$). No differences were found between DEL and ARR treatment for cattle that were treated 1, 2 or 3 times during the feeding period ($P > 0.16$). Immune response to the OVA injections that were given at d 0 and d 28 tended to be different ($P < 0.07$) with the ARR steers having a higher concentration OVA IgG. In steers vaccinated on d 28 which had not received OVA, on d 0 no difference was found between DEL and ARR treatments ($P > 0.49$). These data suggest that delayed implantation did

not impact health or immune response. However, under the conditions of this experiment delaying implanting 28 d did improve overall feed efficacy.

In experiment 2, 124 newly weaned Angus, Hereford, and Angus × Hereford bull and heifer calves (initial BW = 233 ± 14.9 kg) were utilized to evaluate 2 feedlot receiving management strategies at ARDEC on feedlot performance over the first 30 d upon arrival to the feedlot. Cattle were blocked by gender and stratified by BW, breed, and age, and assigned to 1 of 14 pens (8 - 10 head/pen). Pens were then assigned to 1 of 2 dietary treatments. Dietary treatments included: 1) a dried distiller's grain-based total mixed ration (**DDG**) initiated upon arrival, or 2) long-stem grass hay followed by a total mixed ration containing no DDG (**HAY**). Calves receiving the HAY treatment received only grass hay for the first d after arrival, long-stemmed grass hay and total mixed ration combination the following 2 d, and a grain based total mixed ration on d 4. Beginning on d 4, calves across all treatments were fed ad libitum amounts of iso-caloric and iso-nitrogenous diets. Calves were weighed on d 0 and 30, and DMI was determined daily. Initial BW was similar ($P = 0.99$) across treatments; however, d 30 BW was greater ($P < 0.001$) for DDG vs. HAY calves. As a result, ADG was greater ($P < 0.001$) for DDG vs. HAY calves (0.59 vs. 0.41 ± 0.04 kg/d, respectively). Gain-to-feed ratio was greater ($P < 0.05$) for DDG vs. HAY calves (0.22 vs. 0.17± 0.013, respectively), and feed-to-gain ratio tended ($P = 0.05$) to be greater in HAY vs. DDG calves. Daily DMI tended ($P = 0.06$) to be greater in DDG vs. HAY calves (2.70 vs. 2.35 $kg \cdot hd^{-1} \cdot d^{-1}$ ± 0.256, respectively). In summary, providing a DDG-based receiving ration to newly weaned calves upon arrival to the feedlot resulted in greater feed intake, gain, and feed efficiency over a 30 d period than traditional long-stemmed grass hay followed by a non-DDG total mixed ration.

Key words: Dried Distillers Grains, Feedlot, Health, Ovalbumin, and Weaning

INTRODUCTION

Weaning is 1 of the most stressful times in a calf's life. Nutritional strategies can affect calf performance, morbidity, and mortality (Hutcheson and Cole, 1986). Grandin (1997) states that there are 2 types of stress: psychological and physical. Psychological stressors include but are not limited to human interaction, novelty, and physical restraint in a squeeze chute. Physical stressors include but are not limited to standing on a truck, lack of food and water, excess intake of a single nutrient (i.e. S), and ambient temperature (Grandin, 1997). Weaning and receiving cattle into a feedlot are 2 of the most stressful times in a calf's life and for many calves these both events happen at or near the same time. Stress and nutrition are interrelated and both must be considered when developing management strategies to receiving highly stressed calves into a feedlot (Hutcheson and Cole, 1986).

Duff and Galyean (2007) suggest that transportation, marketing, and commingling all have a negative impact on calf immunity. Acute phase protein concentrations in the blood of cattle increase in response to stress (Conner et al., 1988). The acute phase proteins are produced after stimulation from pro-inflammatory cytokines. Pro-inflammatory cytokines have been shown to inhibit growth and increases proteolysis in animals (Johnson, 1997). Activating this immune response for the production of the acute phase proteins increases the animals demand for nutrients specifically protein and to replace lost tissues (Arthington et al., 2005).

The cattle industry has commonly used growth promoting implants for over 55 years to improve ADG and feed conversion (Belk, 1989 and Hancock et al., 1991). Griffin et al. (2009) suggest that more research is needed on strategies for implanting high risk cattle. Due to the lack of research Duff and Galyean (2007) question the effect that implants have on the immune system of highly stressed calves.

Research has shown that hormonal growth promotants increase basal metabolic rate and heart rate; they have also been shown to effect nitrogen excretion (Rumsey et al., 1973 and 1980; Rumsey and Hammond, 1990). Rumsey et al. (1981) and Rumsey (1982) showed that steers in the feedlot increased their protein deposition by more than 20%. This would indicate that nitrogen intake becomes even more crucial for implanted cattle.

It has been well established that stress and nutrition are interrelated; stress can exacerbate any nutritional deficiencies and nutritional deficiencies can initiate a stress response in an animal (NRC, 2000). Nutritional strategies can affect calf performance, morbidity, and mortality (Hutcheson and Cole, 1986). During the first 1 to 3 weeks in a feedlot calves have depressed feed intake (Hutcheson, 1980). After reviewing 18 experiments Hutcheson and Cole (1986) found that not all calves eat daily during the first 2 weeks in a feedlot. Sowell et al. (1998) found a 30% decrease in time at feed bunk for morbid calves, and this was most pronounced in the first 4 days after arrival. Therefore getting calves to eat as soon as possible could reduce morbidity and improve animal well-being, feedlot performance, and profitability of cattle.

Increasing energy demand for growth through the use of growth promotants coupled with a reduction in feed intake in newly received calves would warrant feeding a high concentrate diet. However, NRC (2000) suggests that receiving diets for highly stressed calves should not exceed 25% concentrate and 4% fat. If a high concentrate diet is used, supplementation of hay for the first 3 to 7 days could off-set the negative impacts associated with a high concentrate diet (NRC, 2000).

With the stress associated with transportation and arriving at a feedlot, calves become very susceptible to disease. This coupled with the increased demand in the body for protein and energy created by growth promotant implants and the need for protein and energy to mount an

immune response, cattle that are arriving into a feedlot may have difficulty remaining healthy. Added on top of this increased demand for energy and protein, the reduction of feed intake by calves the first 3 weeks in the feedlot create circumstances conducive for a disease outbreak in the cattle. Research is need to investigate delaying implanting improves calf immunity and health during the feedlot phase.

With the increased stress and exposure to disease agents upon arrival in the feedlot, and the reduction of feed intake in the first 3 weeks of arrival into the feed yard it is very important that receiving diets are formulated to encourage as much feed intake as possible. Also diets must be energy dense to ensure that every kg of feed consumed contains the appropriate amount of energy and protein for that classification of animal. With all of the new by-products available to producers, these products must be evaluated as to their ability to draw calves to the bunk or to depress feed intake.

MATERIALS AND METHODS

Prior to the initiation of this experiment, care, handling, and sampling of animals as described herein were approved by the CSU Animal Care and Use Committee.

Experiment 1: Four hundred and forty two newly weaned Angus and Angus crossbred steers (initial BW = 234 ± 40.4 kg) were selected from an initial group of 453. The steers originated from 3 ranches across Colorado (ECRC, n = 150; Harman Brother's Farms, Otis, CO, n = 200; and Rabbit Creek Ranch, Livermore, CO, n = 92). Upon arrival (ECRC, September 10; Harman Brother's Farms, October 6 and 7; Rabbit Creek Ranch, October 13) to ECRC individual BW was recorded, an individual electronic identification tag was applied, and steers were back poured with an insecticide (Saber™ Pour-On, Merck Animal Health, Whitehouse

Station, NJ) to control external parasites. Steers were then placed into pens, and fed common diets throughout the trial (Table 3.1.). Harmon steers received an oral microbial paste (Loomix, Johnstown, CO) upon request of the owner.

Once all steers from a single ranch were received at ECRC, steers were then ranked by weight within that ranch, and individuals that were beyond ± 3 SD from the mean were removed from the experiment if that ranch had excess steers. In addition, individuals showing health problems were also excluded from the experiment. Remaining steers were assigned a random number from 1 to 1000 using the RAND function of Microsoft® Excel 2003 (Microsoft Inc., Seattle, WA). A sufficient number of steers with the lowest random numbers were removed from further consideration for the experiment to reach the 442 steers required for the experiment. Steers were ranked by weight within ranch; each pair of steers were assigned to treatments 1 or 2 using the lowest to highest random number assigned to the steers, respectively. This was repeated until all steers were assigned to treatment. The lightest 25 or 23 steers per treatment were assigned to a single pen, then the next heaviest 25 or 23 steers per treatment were assigned to a single pen, and this was repeated until all steers were assigned to a pen.

Steers were housed in dirt surfaced pens measuring 12.2 x 42.7 m with a single automatic water fountain shared between every 2 pens. Feed was delivered to steers in fence-line (12.2 m in length) concrete feed bunks allowing for 53 or 48.8 cm (23 and 25 animals per pen, respectively) of linear bunk space per steer and which had a 3.5-m-wide concrete apron adjacent to the feed bunk to provide a solid area for steers to stand while eating. The water fountains were approximately in the middle of one of the 42.7 m fence line of each pen with a 90 cm apron to provide a solid area for steers to stand drinking.

Processing the d that all steers from a ranch arrived at ECRC consisted of collecting an individual BW, vaccinating with a modified live virus (Bovi-Shield Gold FP5 L5 HB®, Pfizer Animal Health, New York, NY), vaccinating with a killed and standardized culture (One Shot Ultra® 8, Pfizer Animal Health), and injecting with an insecticide (Promectin™ VEDCO Inc., Saint Joseph, MO) to control internal and external parasites. If appropriate, implant treatment was also applied at this time. Arrival (**ARR**) treatment received an implant of Revalor®-XS (40 mg estradiol and 200 mg trenbolone acetate; Intervet/Schering-Plough Animal Health, Millsboro, DE). Delayed (**DEL**) treatment received no implant at this time. Steers were given booster vaccinations on d 28 as is standard operating procedure for ECRC. At this time the DEL treatment received a Revalor-XS implant. Individual BW were collected every 28 d for each pen of cattle until d 56 then weights were collected every 56 d.

On d 0, 5 steers from each pen were inoculated with 2 mL of ovalbumin (**OVA**) subcutaneously and 1 mL was administered intradermally. Ovalbumin was used because it is a protein that is novel to the animal and will elicit an immune response without being harmful. This was repeated on d 28 using the same 5 steers from each pen and 5 new steers in each pen. Administering the OVA in this manner allowed for the evaluation of a primary OVA immunoglobulin (**IGg**) response (d 0) and a primary and secondary OVA IGg response following d 28 and for a primary response on d 28. The OVA solution was comprised of 160 mg of crystallized OVA (chicken egg albumin) dissolved in 60 mL phosphate buffered saline (**PBS**, pH 7.4) which was prepared approximately 1 day prior to use. On the day of the inoculation, the OVA and PBS solution was mixed with 60 mL of Freund's Incomplete Adjuvant, this allowed each animal to receive 4,000 ug of OVA.

Blood samples were taken on d 0, 7, 14, 28, 30, 35, 42, 56, 84, 112, 140, 169, and prior to harvest. A 10 mL BD Vacutainer® (Franklin Lakes, NJ 07417) of blood was collected via jugular venipuncture on all steers that were vaccinated with OVA. Blood was collected in both a sodium heparinized tube and a non-treated tube (Franklin Lakes, NJ 07417). Blood was placed on ice after being collected and then was transported back to the laboratory in Fort Collins, CO. Blood samples were spun at 931 x G for 25 min at 4°C. Plasma samples were then collected and frozen at -20°C for ELISA analysis of the OVA IgG.

Animals were harvested in 3 different commercial abattoirs, however, complete blocks were harvested together at the same abattoir and on the same d. Carcass data were collected by the abattoir harvesting the animals and camera data was used for quality and yield grade.

Experiment 2: One hundred and twenty four newly weaned Angus, Hereford, and Angus × Hereford bull and heifer calves (initial BW = 233 ± 14.9 kg) were selected from an initial group of 134 calves. Twenty five days prior to weaning, calves were vaccinated with 7-way clostridial vaccine (UTRABAC® 7, Pfizer Animal Health, New York, NY), modified live virus respiratory vaccine (Bovi-Shield Gold FP5 L5 HB®, Pfizer Animal Health), and BW was collected. No implant was given at any time because the majority of these calves were retained as breeding animals.

Following processing, mean weights were computed for each of the 3 breed and gender classifications. Animals that were beyond ± 2 SD from the mean BW were excluded from the experiment. A random number was assigned to each animal and animals with the lowest random number were excluded from the trial until only 124 animals needed for the experiment remained. Animals were blocked by gender, stratified by breed and BW within gender and randomly assigned to feedlot pens. Treatments were randomly assigned to feedlot pens.

The morning of weaning, cows and calves were gathered from a group pasture in Southeast Wyoming at the CSU's Y-Cross Ranch. Calves were sorted from cows and transported via 2 semi-truck trailers approximately 2 h to CSU's Agricultural Research, Development, and Education Center (Fort Collins, CO). Upon arrival, calves were processed. Processing included a booster of modified live virus vaccine (Bovi-Shield Gold FP5 L5 HB®, Pfizer), BW collection, an identification tattoo in each ear, DNA sample collection, and placement of calves into treatment pens. The experiment was conducted during the months of September and October, and calves were housed in dirt surfaced pens (8 bulls or 10 heifers per pen) measuring 40 x 6.1 m with a single automatic water fountain shared between every 2 pens. Feed was delivered to calves in fence-line (6.1 m in length) concrete feed bunks allowing for 61 or 76.25 cm (heifers and bulls, respectively) of linear bunk space per calf and which had a 3.5-m-wide concrete apron adjacent to the feed bunk and water fountain to provide a solid area for calves to stand while eating or drinking. Thirty days after initiation of the experiment at 1300 h a final BW was collected. A 3% shrink was applied to final BW because calves had ad libitum access to water and 50% of one day's feed delivery prior to obtaining the final weight.

During the experiment, all diets were fed once daily at 0700 h. Feed bunks were evaluated at 1700 h on the previous day and bunks devoid of feed were noted. At 0600 h the next day, bunks were swept and orts were collected and weighed. The target was to have 0.5 to 1.0 kg/pen of feed remaining in the bunk. If bunks were devoid of feed at 1700 or for 2 consecutive mornings; the daily feed amount was increased by 0.45 kg of feed (AF) per calf. When 5 kg or more were remaining in the bunk at 0600 h, the feed call was decreased by the amount remaining in the bunk. Dietary treatments included: 1) a dried distiller's grain-based TMR (**DDG**) initiated upon arrival, or 2) long-stem grass hay followed by a TMR containing no

DDG (**HAY**). Calves receiving the HAY treatment received only grass hay for the first d after arrival, long-stem grass hay and TMR combination the following 2 d, followed by a grain-based TMR on d 4. The proximate analysis of the grass hay on a DM basis was CP 11.96%, ADF 36.39%, and NDF 60.26%. Predicted NEm and NEg was 1.08 and 0.529 Mcal/kg DM. Beginning on d 4, calves across all treatments had access to iso-caloric and iso-nitrogenous diets (Table 3.7).

Statistical Analysis

Experiment 1: The experiment was conducted as a randomized block design with 2 treatments. The treatments (implant timing) were applied to individual animals; therefore, individual animal could be considered as the experimental unit for BW, ADG, and carcass data. However, some data could only be collected at the pen level such as DMI, F:G, and G:F. Furthermore, pen is the most common experimental unit in feedlot research and the most valuable to producers. For these reasons, much of the data were analyzed on both an individual and pen basis to allow the reader to decide which analysis was most appropriate. The experiment was conducted using 18 pens, 9 replicates per treatment and 442 animals, 221 per treatment (25 or 23 animals per pen).

The procedure of PROC MIXED of SAS (Statistical Analysis System, version 9.2, Cary, NC) was used for all analysis of continuous data. Treatment and replicate were included in the models as class variables. Replicate was included in the model as a random variable. Initial body weight was used as a covariate when found to be significant ($P \leq 0.05$) in the model and Kenward-Roger degrees of freedom were computed. Differences between treatment means were examined using the PDIFF option of the LSMEANS statement in SAS. Immunoglobulin data

were analyzed as repeated measures and individual animal was included in the model as a class variable.

Categorical data including quality grade, yield grade and health pulls were evaluated using PROC GLIMMIX in SAS. Treatment and replicate were included in the models as class variables. Replicate was included in the model as a random variable. Differences between treatment means were examined using the PDIFF option of the LSMEANS statement in SAS. Categorical data were only evaluated on a pen basis.

Experiment 2: The experiment was conducted as a randomized block design with 2 treatments and 2 gender blocks with a total of 7 replicates per treatment. Live BW, ADG, G:F, feed to gain ratio, and DMI data were analyzed on a pen mean basis using the PROC MIXED model procedures of SAS (SAS Institute Inc., Cary, NC). Treatment and gender were included in the model as fixed effects. Pen was included in the model as a random effect. Initial BW was included as a covariate when analyzing final BW.

Feedlot performance data were analyzed as a randomized complete block design using PROC MIXED of SAS (Statistical Analysis System, version 9.2, Cary, NC). Diet and sex were included in the models as fixed variables. Pen was included in the model as a random variable. All variables were considered as classification variables. Initial body weight was used as a covariate when analyzing final body weight and Kenward-Roger degrees of freedom were computed. Pen was used as the experimental unit for all data analyzed. Differences between treatment means were examined using the PDIFF option of the LSMEANS statement in SAS.

RESULTS AND DISCUSSION

Experiment 1: Feedlot gain by treatment data are included in Table 3.2. Initial BW was similar ($P = 0.7466$) across treatments. When analyzed on a pen basis d 28 BW were not different ($P > 0.25$); however, when analyzed on an individual basis ARR steer had a tendency ($P = 0.07$) to have heavier BW then DEL steers (293 vs. 291 ± 1.7 kg, respectively). Likewise ADG from d 0 to d 28 tended ($P < 0.07$) for the ARR treatment to have increased ADG over the DEL (1.80 vs. 1.71 ± 0.056 kg, respectively). This is consistent with other research that reported cattle administered a growth promotant gain more live weight then cattle that do not receive one (Duckett and Andrae, 2001, Rumsey et al., 1973 and Samber et al., 1996). By d 56, on an individual and a pen basis, BW was similar ($P > 0.11$) across treatments. Final BW were greatest for the DEL treatment ($P < 0.05$), this resulted in a time by treatment interaction (636 vs. 627 ± 8.3 kg, respectively). This increased final BW is consistent with the overall ADG where DEL treatment out gained ARR (1.78 vs. 1.74 ± 0.029, respectively). This is consistent with Bruns et al. (2005), who compared 3 treatments; no implant, implant on arrival at the feedlot, and delayed implant at d 56. They found an improvement in live BW, ADG, and G:F in the cattle implanted upon arrival in the first 56 d when compared to the other treatments. However, on d 112 there was no difference between the delayed treatment and the implanted upon arrival treatment for live BW and ADG. This can be explained by steers receiving growth promotant being heavier than steers without. The manufacturer of Revlor-XS states that it is a 200 d implant and a representative of the manufacturer stated that company data would suggest that the active life of the implant is no more than 220 d (personal communication). Steers were on feed for between 197 to 236 d therefore, the majority of the steers in the ARR treatment were fed beyond the active life of the implant whereas the DEL treatment steers were harvested during or

right after the active life of the implant. Lighter cattle have a lower maintenance energy requirement and deposit more lean tissue than do heavier cattle closer to harvest weight (NRC, 2000). Therefore, these data would suggest that cattle at the end of the feeding period have greater need for growth promotant then cattle at the beginning of the feeding period.

Steers in the ARR treatment had increased DMI over the DEL treatment from d 0 through d 112 ($P < 0.018$). However, from d 113 through harvest there was no difference ($P > 0.20$) between treatments. Therefore, total DMI tended ($P = 0.052$) to be greatest for the ARR treatment when compared to the DEL (9.81 vs. 9.56 ± 0.194 kg, respectively). Munson et al. (2012) found no difference ($P = 0.40$) in DMI when comparing cattle that had been implanted upon arrival into the feedlot to cattle that received an implant 45 d later. This difference could be due to difference in initial BW where Munson et al. (2012) had heavier steers than were used in the current experiment (274 ± 4.8 vs. 243 ± 13.1, respectively). Suggesting that they had older cattle able to eat more at the start of the feeding period when the difference was greatest in the current experiment. In the current experiment cattle on both treatments were removed from their pens on d 28 and caught in the squeeze chute. This helped to remove any confounding effects of handling stress. However, it is unknown what the impact on response variables would have been were the ARR steers left in their pens and not processed a second time and DEL steers were first processed 28 d after arrival. It can be estimated that by leaving the implanted steers in their pens they would have had a better feed intake for that d and the following d thereby increasing the difference in overall DMI and may have also increase ADG for those cattle and changed the efficiency ratios. Dry matter intake and conversions by treatment is included in Table 3.3.

Two numbers are used when calculating the efficacy of cattle to convert feed into gain, DMI and weight gain. These 2 numbers are used to form a ratio of either feed to gain (**F:G**) or

gain to feed (**G:F**). In this experiment the ARR steers had a higher overall DMI and the DEL steers had a higher overall total BW gain, this would suggest that the ARR treatment should have had a lower G:F and a higher F:Gwhen compared to the DEL treatment. As expected this did happen, ARR steers had lower G:F (0.175 vs. 0.186 ± 0.01) and higher F:G (5.72 vs. 5.40 ± 0.11) when compared to DEL steers ($P < 0.0001$). This is consistent with Bruns et al. (2005) who showed that after 112 d on feed G:F favored the delayed treatment over the implanted upon arrival and the non-implanted cattle. However, Samber et al. (1996) compared 7 implant treatments, a control that received no implant, 2 treatments the received Ralgro® upon arrival at the feedlot, 2 treatments that delayed first implant until d 30 on feed, and 2 treatments that received Revalor-S® upon arrival at the feedlot. Samber et al. (1996) did not find a difference in ADG, G:F, or live BW between steers implanted upon arrival and the steers that were delayed implanted on d 30. Steers that were not implanted did have a lighter final live BW and lower ADG over the entire trial when compared to steers that were implanted. Samber et al. (1996) reported no difference in DMI between control cattle that received no implant and cattle that received an implant suggesting the in the increase in feed conversion is due solely to improvements in ADG. This difference could be due to the use of a more aggressive implant in the current experiment when compare to Samber et al. (1996).

Continues carcass data were evaluated on both a pen and individual basis. On a pen basis, no differences were found however a trend ($P < 0.16$) was found for HCW which would be consistent with DEL treatment having a heavier final BW. When analyzed on an individual basis HCW, was different ($P < 0.0001$) with the DEL have a 6 kg heavier weight the ARR (384 vs. 378 ± 4.7 kg, respectively). Carcass quality grade, yield grade, dressing percentage, and longissimus dorsi area were not different between treatments ($P > 0.4$). This is consistent with

other research that compared cattle implanted upon arrival to cattle implanted later in the feeding period (Samber et al., 1996 and Munson et al., 2012). Carcass data by treatment are included in Tables 3.4 and 3.5.

The health of a pen of cattle can be the difference between profit and loss for a feedlot. Cattle treated for respiratory disease have lower ADG, HCW, and lower quality grades (Gardner, 1999). Due to the demand for protein by the body to mount an immune response and growth promotant directing protein toward muscle deposition and adding to this the reduction in feed intake by most cattle the first d in the feedlot, it was hypothesized that delaying implanting until the cattle were adjusted to the feedlot and onto full feed would improve health and immune response. Table 3.6. demonstrates the effect that timing of implanting had on the number of times cattle were treated for any health issues. No differences were found between DEL and ARR treatment for cattle that were treated 1, 2 or 3 times during the feeding period ($P > 0.16$). Health data are limited for experiments comparing timing of implanting. However, this finding is constant with Munson et al. (2012). They found no differences between cattle implanted upon arrival and cattle implanted 45 d after arrival on response variable of cattle retreated, medicine cost, and mortality rate ($P > 0.3$). To evaluate the steers' ability to mount a primary and secondary immune response OVA injection was given at d 0 and d 28. Figure 3.1., demonstrates the primary and secondary response of OVA IgG. There was a trend ($P < 0.07$) for ARR steers to have a higher OVA IgG concentration then the DEL steers. One possible explanation for this could be the increased DMI of the ARR treatment from d 0 through d 56. On average a calf in the ARR treatment consumed 17 more kg of feed during this period then a calf in the DEL treatment. This equates to 2.25 kg of CP and 17.99 Mcal of NEg. These nutrients would be crucial for a steer to mount an immune response. On d 28 steers which had not received OVA on

d 0 were also given an injection of OVA (Figure 3.2.) and no difference was found between DEL and ARR treatments (P > 0.49).

Experiment 2: Calves used in this experiment were low stress cattle (one source, no co-mingling, haul for less the 2.5 h, and had preconditioning vaccinations) this could explain some of the results of this experiment. Feedlot performance of calves by treatment is included in Table 3.8. Initial BW was similar ($P = 0.99$) across treatments; however, d 30 BW was greater ($P < 0.001$) for calves receiving DDG vs. HAY treatment. As a result, ADG was greater ($P < 0.001$; 0.59 vs. 0.41 ± 0.04 kg/d, respectively) for DDG vs. HAY calves. The greater performance of the DDG treatment could be due to increased energy density of the ration in the first 4 d, and the for increased DMI ($P = 0.06$) over the 30-d period. National Research Council (2000) suggests that newly received calves into the feedlot receiving a higher energy diet demonstrate higher rates. This is also consistent with Fluharty and Loerch (1996) who found calves consuming higher energy diets performed better during the first week after being received into the feedlot.

Gain-to-feed ratio was greater ($P < 0.05$; 0.22 vs. 0.17± 0.013, respectively), and F:G tended ($P = 0.05$) to be greater in HAY vs. DDG calves. Daily DMI tended ($P = 0.06$) to be greater in DDG vs. HAY calves (5.94 vs. 5.17 $kg \cdot hd^{-1} \cdot d^{-1}$ ± 0.256, respectively). Wagner et al. (2012) found no improvement in ADG or DMI when comparing a dry-rolled corn receiving diet to a wet distiller's grain receiving diet.

No calf mortality was observed during the current experiment, which is consistent with Fluharty and Loerch (1996). Also, 1 calf was treated for illness during the 30-d period, which is historically consistent with calves weaned from this cowherd. This would suggest that the cattle used for this experiment were not high stress cattle, and could explain why we didn't see that

advantage to providing long stem grass hay as the NRC (2000) would suggest. This discrepancy may also be explained by the NDF in the DDG due to the high NDF concentration of dried distiller's grains. During the first 4 d of the experiment calves receiving the HAY treatment would have had a higher NDF due to the long stem grass hay being fed.

As shown in Table 3.7., fat concentration in the DDG diet was higher than the fat concentration in the HAY diet (3.46 vs. 5.03, respectively). National Research Council (2000) states that receiving diets should not exceed 4% fat, however, the DDG did exceed this by 1 percentage point and the cattle gained more on the higher fat diet than those cattle on a lower fat diet. Therefore, fat concentration may not be as critical in diets being fed to low stress cattle that are being received into a feedlot.

The results of this experiment are difficult to interpret due to the lack of a TMR without distiller's grains and without grass-hay for the first 4 d. This was due to a limited number of animals available for this experiment. Subsequent experiments should investigate this effect.

IMPLICATIONS

Nutrition, stress, handling, and many other variables can impact feedlot performance of a pen of cattle. All of variables must be considered when managing cattle. This chapter explored 2 management strategies to improve cattle performance upon arrival in a feedlot. Timing of implanting can affect live BW gain and overall feed conversion. Days on feed and active life of the implant must be considered when deciding when to administer implants. If the cattle will be on feed longer than the active life of the implant delaying implanting may be a way to reduce cost associated with multiple implants without sacrificing gain or efficiency. Under the

conditions of this experiment timing of implant did not affect immune response, overall health of the cattle or carcass quality.

A starting diet can impact cattle the overall performance of cattle in a feedlot and influence the health of the cattle consuming the diet. Under the circumstance of this experiment, relatively low stress and healthy calves, the performance advantage is to an energy dense diet. The DDG diet improved ADG and live BW over the first 30 d on feed and did not negatively impact the health of the cattle. Neutral detergent fiber may be a key to balancing a starter ration and this may be why dry distiller's grains are successful in these rations.

Table 3.1. Ingredient and theoretical nutrient concentration for the starter, step-up, and finishing diets used for the delayed implant experiment.

Item[a]	Starter	Step-one	Step-two	Step-three	Finish
Ingredient[b]					
Cracked corn	25.00	25.00	37.24	47.40	50.93
Alfalfa hay	21.51	2.59	0.00	0.00	0.00
DDG[c]	19.39	25.95	30.00	29.50	35.00
Triticale hay	31.85	43.96	29.76	20.98	10.57
Supplement[d]	2.25	2.50	3.00	2.13	3.50
Nutrient					
Dry matter, % of as-fed	86.69	87.16	87.13	86.69	87.05
Crude protein	13.25	13.25	13.93	14.04	15.04
Non-protein nitrogen	0.13	0.14	1.17	0.12	0.20
NEm, Mcal/45.35 kg DM	77.51	84.64	91.77	93.61	97.54
NEg, Mcal/45.35 kg DM	48.50	54.50	60.50	62.33	65.50
Calcium	0.74	0.56	0.55	0.40	0.57
Phosphorus	0.34	0.37	0.39	0.39	0.41
Potassium	1.13	1.09	0.93	1.01	0.84
Magnesium	0.26	0.25	0.23	0.21	0.21
Sulfur	0.27	0.29	0.29	0.32	0.33
Vitamin A, IU/45.35 kg DM	1687.11	1864.49	2238.14	1228.44	2055.06
Vitamin E, IU/45.35 kg DM	1.95	2.15	2.58	1.20	2.01

[a] Percentage of dry matter unless stated otherwise.
[b] As-fed basis.
[c] Dried distiller's grains.

Table 3.2. Least square means showing the effect of implant timing on live body weight and ADG on an individual steer and pen basis.

	Implant			
Item[a]	Arrival	Delay	SEM	P-Value
Individual Basis				
Body Weight				
Initial, kg	243	243	13.1	0.7466
D 28, kg	293	291	1.7	0.0685
D 56, kg	342	340	1.9	0.1131
D 112, kg	442	441	4.1	0.7547
D 169, kg	538	543	4.6	0.1179
Final, kg	627	636	8.3	0.0112
ADG				
Period 1, kg	1.80	1.71	0.056	0.0720
Period 2, kg	1.76	1.76	0.073	0.9684
Period 3, kg	1.78	1.81	0.048	0.2843
Period 4, kg	1.70	1.80	0.059	0.0027
Period 5, kg	1.76	1.83	0.103	0.0900
Overall, kg	1.74	1.78	0.029	0.0115
Pen Basis				
Body Weight				
Initial, kg	243	243	13.1	0.6075
D 28, kg	294	291	1.8	0.2530
D 56, kg	343	341	2.0	0.1587
D 112, kg	442	442	4.4	0.7915
D 169, kg	539	543	4.3	0.1066
Final, kg	627	637	4.8	0.0215
ADG				
Period 1, kg	1.80	1.71	0.061	0.2528
Period 2, kg	1.76	1.76	0.086	0.9186
Period 3, kg	1.78	1.81	0.053	0.6204
Period 4, kg	1.70	1.81	0.059	0.0135
Period 5, kg	1.76	1.84	0.121	0.3755
Overall, kg	1.74	1.78	0.032	0.0256

[a] D 28 = Day 28; D 56 = Day 56; D 112 = Day 112; D 169 = Day 169; ADG = Average Daily Gain ($kg \cdot hd^{-1} \cdot d^{-1}$); Period 1 = Day 0 through day 28; Period 2 = Day 29 through day 56; Period 3 = Day 57 through day 112; Period 4 = Day 113 through day 169; Period 5 = Day 170 through day Harvest; Overall = ADG for day 0 through day harvest

Table 3.3. Least square means showing the effect of implant timing on dry matter intake and feed conversion.

	Implant			
Item[a]	Arrival	Delay	SEM	P-Value
DMI, kg				
Period 1	6.68	6.39	0.247	0.0179
Period 2	8.42	7.92	0.288	0.0116
Period 3	9.84	9.36	0.293	0.0188
Period 4	10.60	10.41	0.188	0.2038
Period 5	11.30	11.37	0.183	0.6378
Overall	9.81	9.56	0.194	0.0518
Feed to Gain				
Period 1	3.7899	3.7478	0.2252	0.8240
Period 2	4.8732	4.5041	0.1902	0.1679
Period 3	5.5578	5.2345	0.2211	0.0154
Period 4	6.3185	5.8231	0.2330	0.0063
Period 5	7.0350	6.6006	0.4495	0.0070
Overall	5.7153	5.4007	0.1018	0.0001
Gain to Feed				
Period 1	0.2727	0.2705	0.01342	0.8414
Period 2	0.2099	0.2226	0.008433	0.2738
Period 3	0.1821	0.1937	0.007464	0.0144
Period 4	0.1602	0.1736	0.006261	0.0062
Period 5	0.1464	0.1566	0.008920	0.0284
Overall	0.1754	0.1857	0.003316	0.0001

[a] Period 1 = Day 0 through day 28; Period 2 = Day 29 through day 56; Period 3 = Day 57 through day 112; Period 4 = Day 113 through day 169; Period 5 = Day 170 through day Harvest; Overall = Day 0 through day harvest

Table 3.4. Least square means describing the effect of implant timing on carcass characteristics reported on an individual steers and pen basis.

	Implant			
Item[a]	Arrival	Delay	SEM	P-value
Individual Basis				
HCW, kg	378	384	4.7	0.0001
DP, %	62.84	62.81	0.320	0.8245
Fat, cm	1.51	1.52	0.032	0.8175
Marbling	482.1	480.4	9.01	0.8550
LDA, cm^2	79.45	80.16	1.155	0.4072
Cal YG	3.61	3.63	0.046	0.7217
Pen Basis				
HCW, kg	378	383	3.6	0.1584
DP, %	62.85	62.80	0.331	0.8181
Fat, cm	1.51	1.52	0.031	0.8317
Marbling	482.0	480.4	9.72	0.9026
LDA, cm^2	79.52	80.08	0.031	0.6171
Cal YG	3.61	3.63	0.043	0.7330

[a]HCW = hot carcass weight; DP = dressing percentage; LDA = Longissimus dorsi area; Marbling = Marbling score units, 400 = $Small^{00}$, 500 = $Modest^{00}$; Cal YG = yield grade calculated from carcass measurements.

Table 3.5. Least square means describing the effect of implant timing on carcass quality grade and yield grade.

Item[ab]	Implant		SEM	P-value
	Arrival	Delay		
USDA Quality Grade				
Standard [c]	0.00	0.93	0.66	
Select	18.57	19.63	2.72	0.7163
Choice^{-}	40.00	36.45	3.39	0.6596
Choice^{0}	30.48	29.44	3.18	0.9170
Choice^{+}	7.62	8.88	1.95	0.6908
Prime	3.33	4.67	1.45	0.4582
Ch & Pr	81.43	79.44	2.77	0.9107
USDA Yield Grade				
YG 1 or 2	16.19	15.42	2.55	0.9161
YG 3	56.19	53.34	3.43	0.7777
YG 4 or 5	27.62	30.84	3.16	0.4072

[a]YG = yield grade Ch & Pr = Sum of Choice^{-}, Choice^{0}, Choice^{+}, and Prime.
[b]Likelihood of an individual qualifying for a specific category.
[c]Too few qualified for each category.

Table 3.6. Least square means describing the effect of implant timing on the number of health treatments.

	Implant			
Item[ab]	Arrival	Delay	SEM	P-value
Pull	21.72	20.74	2.78	0.7138
Repull	2.71	5.53	1.56	0.1632
Third Pull	0.45	0.92	0.65	0.6640

[a] Pull = Treated 1 time for health issue; Repull = Treated 2 times for health issues; Third Pull = Treated for health issues 3 times.
[b]Likelihood of an individual qualifying for a specific category.

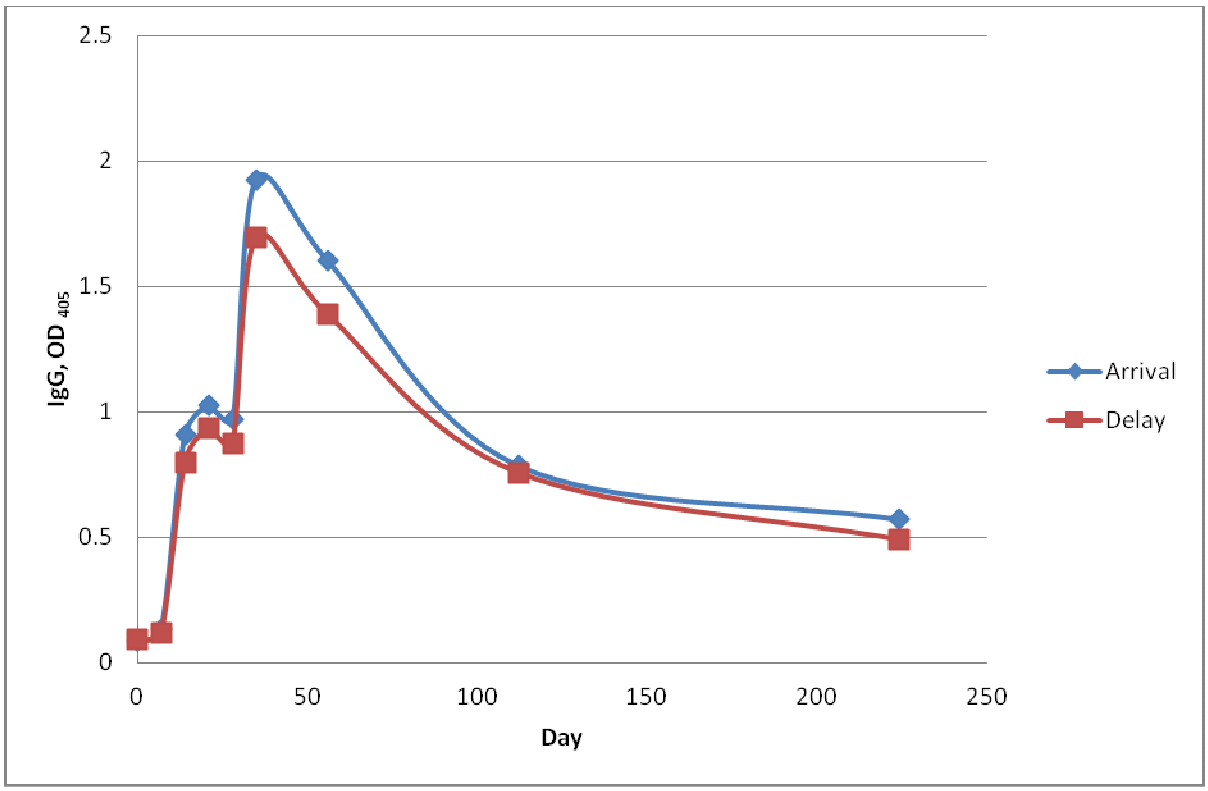

Figure 3.1. Day 0 primary and 28 secondary Ovalbumin immunoglobulin response to implant timing.

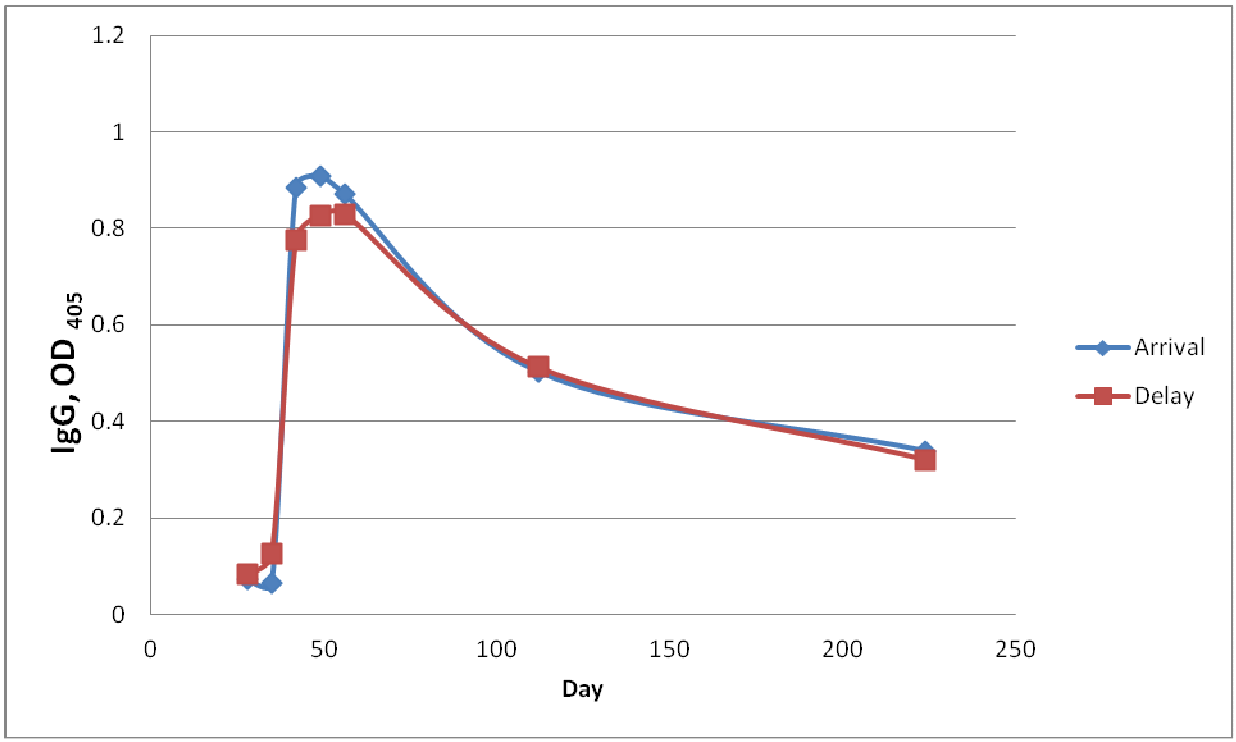

Figure 3.2. Day 28 primary Ovalbumin immunoglobulin response to implant timing.

Table 3.7. Ingredient composition of diets for both treatments on a DM basis

	Diet	
Item[1]	HAY[2]	DDG[3]
Corn Silage	28.69	29.06
Cracked Corn	28.67	31.34
Wheat Straw	3.78	13.29
Alfalfa hay	36.53	0.00
Dry Distiller's Grains	0.00	22.73
Calcium Carbonate	0.00	1.25
Supplement	2.33	2.33
DM, % AF	60.01	60.04
CP	14.50	14.50
NPN[4]	1.09	1.09
NDF	31.24	35.60
NEm, Mcal/kg	1.62	1.64
NEg, Mcal/kg	1.06	1.06
Fat	3.46	5.03
Calcium	0.68	0.75
Phosphorus	0.29	0.38
Ca:P ratio	2.36	1.96

[1] Percentage of DM unless stated otherwise.
[2]HAY treatment received only grass hay for the first d after arrival, long stem grass hay and total mixed ration combination the following 2 d. Beginning on d 4, calves across all treatments had access to iso-caloric and iso-nitrogenous diets.
[3] DDG treatment received only the total mixed ration for the entire 30-d period.
[4]CP equivalent.

Table 3.8. Feedlot performance of beef calves comparing receiving strategies at weaning

	Treatment			
Item	HAY[1]	DDG[2]	SEM	Prob. > F
Initial BW, kg	233	233	14.9	0.99
Final BW, kg	258	269	1.2	0.001
ADG, $kg \cdot hd^{-1} \cdot d^{-1}$	0.41	0.59	0.04	0.001
DMI, $kg \cdot hd^{-1} \cdot d^{-1}$	5.17	5.94	0.256	0.06
G:F	0.17	0.22	0.013	0.05
Feed to gain	5.86	4.72	0.376	0.05

[1] HAY treatment received only grass hay for the first d after arrival, long stem grass hay and total mixed ration combination the following 2 d. Beginning on d 4, calves across all treatments had access to iso-caloric and iso-nitrogenous diets.
[2] DDG treatment received only the total mixed ration for the entire 30-d period.

LITERATURE CITED

Arthington, J. D., J. W. Spears, and D. C. Miller. 2005. The effects of early weaning on feedlot performance and measures of stress in beef calves. J. Anim. Sci. 83:933-939.

Belk, K. E., H. R. Cross, and D. Nash. 1989. Effects of trenbolone acetate, estrogens on steer explored. Feedstuffs 61(50):15.

Bruns, K. W., R. H. Pritchard, and D. L. Boggs. 2005. The effect of stage of growth implant exposure on performance and carcass composition in steers. J. Anim. Sci. 83:108-116.

Conner, J. G., P. D. Eckersall, A. Wiseman, T. C. Aitchison, and T. A. Douglas. 1988. Bovine acute phase response following turpentine injection. Res. Vet. Sci. 44:82-88.

Duckett, S. K. and J. G. Andrae, 2001. Implants strategies in an integrated beef production system. J. Anim. Sci. 85:823-840.

Duff, G. C., and M. L. Galyean. 2007. Board-invited review: Recent advances in management of highly stressed, newly received feedlot cattle. J. Anim. Sci. 85:823-840.

Fluharty, F. L., and S. C. Loerch. 1996. Effects of dietary energy source and level on performance of newly arrived feedlot calves. J. Anim. Sci. 74:504-13.

Gardner, B. A., H. G. Dolezal, L. K. Bryant, F. N. Owens, and R. A. Smith. 1999. Health of finishing steers: Effects of performance, carcass traits and meat tenderness. J. Anim. Sci. 77:3168-3175.

Grandin, T. 1997. Assessment of stress during handling and transport. J. Anim. Sci. 75:249-257.

Handcock, D. L., J. F. Wagner, and D. B. Anderson. 1991. Effects of estrogens and androgens on animal growth. Pages 197-255 in growth regulation in farm animals: Advances in meat research. Volume 7. A.M. Pearson and T.R. Dutson, eds. Elsevier Appl. Sci., New York, NY.

Hutcheson, D. P. 1980. Observations on receiving new cattle. Texas Beef Conf., Amarillo, TX. April.

Hutcheson, D. P., and N. A. Cole. 1986. Management of transit –stress syndrome in cattle: nutritional and environmental effects. J. Anim. Sci. 62:555-560.

Johnson, R. W. 1997. Inhibition of growth by pro-inflammatory cytokines: An integrated review. J. Anim. Sci. 75:1244-1255.

Munson, R. D., D. U. Thomson, and C. D. Reinhardt. 2012. Delayed implant in high risk calves has no benefit for health or feedlot performance. K. State; Report of progress. 1065:25-28.

NRC. 1984. Nutrient Requirements of Beef Cattle. Sixth Revised Edition. National Academy Press. Washington, DC.

NRC. 2000. Nutrient Requirements of Beef Cattle. Seventh Revised Edition. National Academy Press. Washington, DC.

Rumsey, T. S., R. R. Oltjen, H. Swan, and D. J. Bolt. 1973. Physiological changes and blood lactic acid values in beef steers fed DES under ad libitum, restricted and compensatory conditions. J. Anim. Sci. 37:1201-1206.

Rumsey, T. S., H. F. Tyrrell, and P. W. Moe. 1980. Effect of diethylstilbestrol and synovex-s on fasting metabolism measurements of beef steers. J. Anim. Sci. 50:160-166.

Rumsey, T. S., H. F. Tyrrell, D. A. Dinius, P. W. Moe, and H. R. Cross. 1981. Effect of diethylstilbestrol on tissue gain and carcass merit of feedlot beef steers. J. Anim. Sci. 53:589-600.

Rumsey, T. S. 1982. Effect of synovex-s implants and kiln dust on tissue gain by feedlot beef steers. J. Anim. Sci. 54:1030-1039.

Rumsey, T. S, and A. C. Hammond. 1990. Effect of intake level on metabolic response to estrogenic growth promoters in beef steers. Anim. Sci. 68:4310-4318.

Samber, J. A., J. D. Tatum, M. I. Wray, W. T. Nichols, J. B. Morgan, and G. C. Smith. 1996. Implant program effects on performance and carcass quality of steer calves finishing for 212 days. J. Anim. Sci. 74:1470-1476.

Sowell, B. F., J. G. P. Bowman, H. W. Sherwood, M. E. Branine, and M. E. Hubbert. 1998. Radio frequency technology to measure feeding behavior and health of feedlot steers. Appl. Anim. Behav. 59:275-282.

Statistical Analysis System. 2003. The SAS System for Windows. Release 9.3.1. SAS Institute Inc. Cary, NC.

APPENDIX

Chapter 2;

In vitro (Experiment 1):

```
proc glimmix;
class bottle run diet sample;
model h2sDMD= sample|diet;
lsmeans sample|diet/slicediff=diet cl;

proc glimmix;
class bottle run diet sample;
model ph= sample|diet run(diet) sample*run(diet);
lsmeans sample*diet run(diet)/slicediff=diet cl;

proc glimmix;
class bottle run diet sample;
model adjtotalh2s= sample|diet run(diet) sample*run(diet);
lsmeans sample|diet run(diet)/slicediff=diet cl;
```

In situ (Experiment 2):

```
proc mixed;
where sample ne 'CCDS' and time ne 0;
class diet steer Sample time;
model adjSlostadjdmd = diet time sample diet*sample;
lsmeans diet time sample sample*diet/ pdiff cl;

proc mixed;
where sample ne 'CCDS' and time ne 0;
class diet steer Sample time;
model perlost= diet|steer|time|sample;
lsmeans diet time sample diet*sample*time/ pdiff cl;

proc mixed;
where sample ne 'CCDS' and time ne 0;
class diet steer Sample time;
model peradjSlost= diet steer time sample diet*steer diet*sample steer*time;
lsmeans diet time sample/ pdiff cl;

proc mixed;
where sample ne 'CCDS' and time ne 0;
class diet steer Sample time;
model adjSlost= diet steer time sample diet*steer diet*time diet*sample steer*sample
time*sample diet*steer*sample diet*sample*time;
lsmeans diet time sample/ pdiff cl;
```

Chapter 3;

Feedlot Performance (Experiment 1):

Individual Basis

```
proc mixed data=penadg;
class trt rep;
model inwght=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model wght28=inwght trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model wght56=inwght trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model wght112=inwght trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model wght169=inwght trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model fnwght=inwght trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model p1=trt/s ddfm=kr;
```

```
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model p1=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model p2=inwght trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model p3=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model p4=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model p5=inwght trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model tadg=inwght trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
```

Pen Basis

```
proc sort data=adg; by pen;
proc means noprint; by pen; var inwght wght28 wght56 wght112 wght169 fnwght p1 p2 p3 p4
p5 tadg dof trt blk rep;
output out=penadg mean=inwght wght28 wght56 wght112 wght169 fnwght p1 p2 p3 p4 p5 tadg
dof trt blk rep;
```

```
proc sort; by pen;
proc print;
proc print data=penadg;

proc mixed data=penadg;
class trt rep;
model inwght=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model wght28=inwght trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model wght56=inwght trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model wght112=inwght trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model wght169=inwght trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model fnwght=inwght trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model p1=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
```

```
proc mixed data=penadg;
class trt rep;
model p2=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model p3=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model p4=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model p5=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=penadg;
class trt rep;
model tadg=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=dmi;
class trt rep ;
model dmi2=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=dmi;
class trt rep ;
model fg2=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=dmi;
class trt rep ;
model gf2=trt/s ddfm=kr;
random rep;
```

```
lsmeans trt/cl pdiff;

proc mixed data=dmi;
class trt rep ;
model dmi3=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=dmi;
class trt rep ;
model fg3=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=dmi;
class trt rep ;
model gf3=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=dmi;
class trt rep ;
model dmi4=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=dmi;
class trt rep ;
model fg4=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=dmi;
class trt rep ;
model gf4=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=dmi;
class trt rep ;
model dmi5=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=dmi;
class trt rep ;
```

```
model fg5=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=dmi;
class trt rep ;
model gf5=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=dmi;
class trt rep ;
model dmi6=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=dmi;
class trt rep ;
model fg6=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=dmi;
class trt rep ;
model gf6=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=dmi;
class trt rep ;
model dmit=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=dmi;
class trt rep ;
model fgt=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=dmi;
class trt rep ;
model gft=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
```

Continuous Carcass Data (Experiment 1):

Individual Basis

```
proc mixed data=car;
class trt rep;
model hcw=inw trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=car;
class trt rep;
model ms=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=car;
class trt rep;
model rea= trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=car;
class trt rep;
model aft=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=car;
class trt rep;
model cyg= trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=car;
class trt rep;
model dp=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
run;
```

Pen Basis

```
proc mixed data=pencar;
class trt rep;
model hcw=inw trt/s ddfm=kr;
```

```
random rep;
lsmeans trt/cl pdiff;

proc mixed data=pencar;
class trt rep;
model ms=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=pencar;
class trt rep;
model rea=inw trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=pencar;
class trt rep;
model aft=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=pencar;
class trt rep;
model cyg= trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;

proc mixed data=pencar;
class trt rep;
model dp=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
run;
```

Categorical Carcass Data (Experiment 1):

```
proc glimmix data=pencat;
class rep trt;
model ch/denom= trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;

proc glimmix data=pencat;
class rep trt;
model ch_/denom= trt/s ddfm=kr error=binomial link=logit;
random rep;
```

```
lsmeans trt/cl pdiff ilink;

proc glimmix data=pencat;
class rep trt;
model ch_0/denom= trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;

proc glimmix data=pencat;
class rep trt;
model pr/denom= trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;

proc glimmix data=pencat;
class rep trt;
model chpr/denom= trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;

proc glimmix data=pencat;
class rep trt;
model highq/denom= trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;

proc glimmix data=pencat;
class rep trt;
model se/denom= trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;

proc glimmix data=pencat;
class rep trt;
model st/denom= trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;

proc glimmix data=pencat;
class rep trt;
model yg12/denom= trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;

proc glimmix data=pencat;
class rep trt;
```

```
model yg3/denom= trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;

proc glimmix data=pencat;
class rep trt;
model yg45/denom= trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;
run;
```

Categorical Health data (Experiment 1):

```
proc glimmix data=hate;
class rep trt;
model pull/denom= trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;

proc glimmix data=hate;
class rep trt;
model repull/denom= trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;

proc glimmix data=hate;
class rep trt;
model tpull/denom= trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;
run;
```

IgG Response (Experiment 1):

```
proc mixed scoring=2;
class ID trt time;
model avg= d0 plate trt time trt*time/ddfm=kenwardroger;
repeated /subject=ID(trt) type=ar(1) r rcorr;
lsmeans  trt time trt*time/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1;

proc mixed scoring=2;
class ID trt time;
model avg= plate trt time trt*time/ddfm=kenwardroger;
repeated /subject=ID(trt) type=ar(1) r rcorr;
lsmeans  trt time trt*time/pdiff;
```

```
contrast 'trt 1 vs trt 2' trt 1 -1;

proc mixed scoring=2;
class ID trt time;
model avg= trt time trt*time/ddfm=kenwardroger;
repeated /subject=ID(trt) type=ar(1) r rcorr;
lsmeans  trt time trt*time/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1;

proc mixed scoring=2;
class ID trt time;
model avg= trt time/ddfm=kenwardroger;
repeated /subject=ID(trt) type=ar(1) r rcorr;
lsmeans  trt time/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1;
```

Feedlot Performance (Experiment 2):

```
proc mixed scoring=2;
class pen trt sex;
model adg= trt sex/ddfm=kenwardroger;
random pen;
lsmeans  trt/pdiff;

proc mixed scoring=2;
class pen trt sex;
model fg= trt sex/ddfm=kenwardroger;
random pen;
lsmeans  trt/pdiff;

proc mixed scoring=2;
class pen trt sex;
model gf= trt sex/ddfm=kenwardroger;
random pen;
lsmeans  trt/pdiff;

proc mixed scoring=2;
class pen trt sex;
model inw= trt|sex/ddfm=kenwardroger;
random pen;
lsmeans  trt/pdiff;

proc mixed scoring=2;
class pen trt sex;
model outw= trt inw sex/ddfm=kenwardroger;
random pen;
```

```
lsmeans  trt/pdiff;

proc mixed scoring=2;
class pen trt sex;
model gain= trt sex/ddfm=kenwardroger;
random pen;
lsmeans  trt/pdiff;

proc mixed scoring=2;
class pen trt sex;
model dmi= trt sex/ddfm=kenwardroger;
random pen;
lsmeans  trt/pdiff;
```