LEVEL OF DISTILLER'S GRAIN WITH SOLUBLE EFFECTS ON GAS EMISSIONS FROM GROWING STEERS

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ABSTRACT

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A rising concern with feeding high levels of distiller's grain with soluble (DGS) is its high S and N content and the effects it might have on S- and N-containing emissions from gas produced in the rumen and manure. Two trials were conducted with 12 Holstein steers housed in individual environmentally-controlled rooms to monitor gas emissions. Three dietary treatments were fed in trial 1; containing 0% (control), 40%, and 60% DDGS. In trial 2, treatments were the same except the 60% DDGS dietary treatment was replaced with a 40% DDGS diet fortified with 8 ppm Mo and 90 ppm Cu, which will be referred to as 40% DDGS+. The 40% DDGS+ diet served as a potential strategy to mitigate S-containing gas emissions. Each trial was divided into 2 phases; phase 1 of each trial monitored emissions when urine and feces were collected in the same vessel. Phase 2 of each trial monitored emissions while steers were fitted with fecal bags to separate feces from urine. Distiller's grain with soluble increased H_2S and NH_3 production ($P < 0.05$) and these emissions were decreased to undetectable levels during phase 2 of each trial compared to emissions generated in phase 1 ($P < 0.01$). Addition of Mo and Cu in trial 2 tended to decrease H_2S emissions when adjusted for S-intake ($P = 0.08$). In trial 2, the 40% DDGS+ treatment decreased CO_2 emissions ($P \leq 0.05$) and tended to generate less CH_4 emissions compared to the control and the traditional 40% DDGS diet.

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LIST OF ABBREVIATIONS

AAQRF……Animal Air Quality Research Facility

ACH…….air changes per hour

ADG……..……average daily gain

BCTRC……..…Beef Cattle Teaching Research Facility

BW……body weight

CDS……..condensed distiller's with soluble

CGM……..……corn gluten meal

CP…….crude protein

CS…….corn silage

DE…….…...digestible energy

DGS…….……distiller's grain with soluble

DDGS……..…dried distiller's grain with soluble

DM……...dry matter

DMI……..dry matter intake

DPD……..*N*, *N*-dimethyl-*p*-phenylenediamine

DTRC…….…..Dairy Teaching Research Facility

FE……fecal energy

G:F……...gain to feed ratio

GI……...…..gastro-intestinal

GHG…….……greenhouse gas

GWP…….…....global warming potential

HCl……...hydrochloric acid

HE……....heat energy

HMC……high moisture corn

IE……gross energy intake

IVDMD……...in-vitro dry matter disappearance

MB……...methylene blue

MS……....mineral supplement

MWDGS……..modified wet distiller's grain with soluble

NEg……..net energy gained or retained energy

NI……nitrogen intake

N_t…….….total nitrogen

NMTHC……...non-methane total hydrocarbon

PEM……polioencephalomalacia

PUFA……...polyunsaturated fatty acids

SI……..sulfur intake

SBM……soybean meal

SRB……..sulfate-reducing bacteria

TCA……tri-carboxylic acid

TMR……total mixed ration

TPP……...thiamine pyrophosphate

TRS…….….total reduced sulfur

TRT……..treatment

UE……urine energy

UV……ultraviolet

VFA……..volatile fatty acid

VOC…….volatile organic compound

WDG…….....wet distiller's grain

WDGS……..wet distiller's grain with soluble

CHAPTER 1: REVIEW OF LITERATURE

BACKGROUND ON DISTILLER'S GRAIN WITH SOLUBLE

For centuries, fermentation of cereal grains has been used to produce beverage alcohol (Klopfenstein, 2008). A co-product from the fermentation process, distiller's grains plus soluble (**DGS**) has been used as a feedstuff in beef cattle diets as early as the 19th century (Henry, 1900; Klopfenstein, 2008). Little was known about the effectiveness and nutrient composition of the co-product feed, DGS when it was first being used as a feedstuff in beef cattle diets. Recently, increased production of ethanol has led to a surplus of DGS. The Renewable Fuels Association (2006) reported that ethanol production in the United States alone was expected to increase by nearly 25% over the previous year, reaching 4.9 billion gallons. At the current rate, production has already exceeded the 2012 target of 7.5 billion gallons per year set forth by the Energy Policy Act of 2005 (Renewable Fuels Association, 2006; Depenbusch, 2008). According to the Renewable Fuels Association (2010), ethanol production is currently greater than 13 billion gallons per year. The availability and cost benefits of feeding DGS have made it a very popular energy and protein replacement over traditional corn and soybean meal (**SBM**) in diets. Extensive research has provided a better understanding of nutrient composition and feeding strategies of DGS.

Dry Milling Process

To produce the co-product DGS, corn or other cereal grains are used for ethanol production. In the typical dry milling process, the entire corn kernel is used and the starch is converted into ethanol during the fermentation process (Bothast and Schilicher, 2005). The basic steps involved in the dry-grind milling process include cleaning and grinding corn to a flour or fine grind meal. The combination of the resulting flour and water form a mash. Enzymes are

added to the mash to breakdown the starch in the kernel to glucose molecules. The mash is then cooked to reduce undesirable bacteria. After the mash is cooked it is then allowed time to cool, during this process yeasts are added to the mash to aid the conversion of glucose to ethanol and carbon dioxide. Next, a distillation process extracts the ethanol and the remaining water and solids are referred to as whole stillage. Typically whole stillage is centrifuged to separate the liquid from the solids. The liquid portion is considered distiller's soluble or thin stillage. The thin stillage can be concentrated in an evaporator to become condensed distiller's with soluble (**CDS**), also referred to as syrup. The solid portion separated out is considered wet distiller's grains (**WDG**). The combination of CDS and WDG form wet distiller's grains with soluble (**WDGS**) and can be dried to produce dried distiller's grain with soluble (**DDGS**) (Kalscheur and Garcia, 2008).

DGS Composition

Distiller's grain with soluble is an excellent source of protein, fat, digestible fiber, and minerals, all of which can be utilized in ruminant diets (Botheast and Schlicher, 2005). The beef NRC (2001) lists crude protein (**CP**) at 29.7% for DDGS. Kalscheur and Garcia (2008) report studies illustrating that CP ranges from 27 to 34%. Roughly 2/3 of the corn kernel is starch, which is removed during the dry milling process. The result of this process causes DGS to be roughly 3-fold higher in protein, fat, fiber, and mineral concentrations. Protein increases from 10 to 30%, fat from 4 to 12%, NDF from 12 to 36%, and P from 0.3 to 0.9% of DM (Stock et al., 2000). The solids fraction of DGS is greater in CP and crude fiber compared to the CDS fraction, but the CDS fraction is greater in fat and minerals in comparison to the solids fraction (Rausch and Belyea, 2006; Kalsheur et al., 2008). Sulfuric acid is added during the fermentation process to regulate pH, which increases the amount and concentration of S in DGS as well (Vannes et al.,

2009; Kelzer et al., 2010). Buckner et al. (2008) reported S concentrations in DGS ranging from 0.44% to as high as 1.5%. Sulfur concentration should be closely monitored with mid to high levels of DGS added to cattle diets as the NRC (2000) lists maximum tolerable concentration in feedlot cattle rations at 0.4%.

FEEDING DISTILLER'S GRAIN WITH SOLUBLE TO FEEDLOT CATTLE

Inclusion levels for DGS

Increased levels of DGS in cattle diets decrease starch intake and in most cases increase levels of CP and fat in feedlot diets. Feeding elevated levels of DGS to finishing cattle is thought to negatively affect feedlot performance and carcass quality (Gunn et al., 2009). Decreased dietary starch content may lead to decreased carcass yield grade and marbling score (Smith and Crouse, 1984). Data from a study conducted by Gunn et al (2009) indicated that live performance, marbling scores, quality grades, and color stability of ground product during retail display were negatively affected when DDGS increased from 25 to 50% of the diet (DM basis). Gunn and others (2009) concluded that this response was related not only to increased CP or fat concentrations within the diet of steers fed elevated levels of DDGS, but also to a combination effect of both CP and fat within these diets. Larson et al. (1993), Ham et al. (1994), Jones (2007), and Depenbusch (2009) reported that moderate levels of DGS in feedlot diets improved ADG, DMI, and G:F. However, all of these studies were conducted with inclusion of either WDGS or DDGS at or below 40% (DM basis). Very little research has evaluated DGS levels above 40% (DM basis) and the effects on growth performance and carcass characteristics (Farlin, 1981; Vander Pol et al., 2006). Gordon et al. (2002) reported a decrease in performance of finishing heifers when DDGS inclusion levels exceeded 45% of dietary DM. Few studies have looked at effects of distillers grains on meat quality (Kroger et al., 2004; Roeber et al., 2005; Gill et al., 2008). A report from Corah and McCully (2006) indicated a decrease in marbling scores when DGS were fed at greater than 30% of DM and an increase in yield grade at all inclusion levels. A study conducted by Roeber et al. (2005) determined that steaks from Holstein steers fed either WDGS or DDGS at 40% dietary DM had less acceptable retail display compared to steers fed

lower concentrations of DGS. Steaks from Holstein steers fed moderate inclusion levels of DGS, around 25% of DM basis had greater redness than steaks from steers fed 0 or 50% DGS (Roeber et al., 2005). Depenbusch et al. (2009) reported that increasing levels of DGS from 0 to 75% increased concentrations of linoleic acid (18:2n-6cis), total n-6 fatty acids, and total polyunsaturated fatty acids (**PUFA**) in cooked ribeye. In addition, myofibrillar tenderness and overall tenderness increased linearly with the inclusion of DGS from 0 to 75% of the diet DM (Depenbusch et al., 2009).

Variability of DGS

Variability in nutrient content that exists among different sources of DGS is one of the challenges for utilization in livestock diets (Spiehs et al., 2002). Studies from Holt and Pritchard (2004) and Kaiser (2005) would suggest that the composition of WDGS can have considerable variance. There are several factors that contribute to this variability but the ratio of distiller's grain and CDS used to manufacture commercial DGS is thought to be the major contributor to nutrient variability among different ethanol plants (Kleinschmit et al., 2007; Cao et al., 2009). Distiller's grain with soluble is comprised of 2 co-products, the spent grain or solids fraction and the CDS fraction. When more of the CDS fraction is added back to the grain fraction to form DGS, fat and mineral concentrations are increased. In comparison, DGS that has a higher grain fraction with less CDS added back will likely be higher in CP and crude fiber (Rausch and Belyea, 2006; Kalcheur et al., 2008; Cao et al., 2009). In addition to distiller's grain to CDS ratio, processing methods can influence variability of nutrients in DGS as well. Sulfur concentration of DGS is highly variable within and among ethanol plants. Variability of S concentration (0.44 to 1.5%; Buckner et al., 2008) is most likely influenced by the inclusion of

sulfuric acid (Vannes et al., 2009; Kelzer et al., 2010) to control pH during fermentation and for use as a cleaning agent.

Effects of feeding wet vs. dry DGS

Dried distiller's grain with soluble is a more costly co-product compared to WDGS, due to the additional expense associated with the drying process. Traditional WDGS contains 30-35% DM and is similar in nutrient content on a DM basis to DDGS (Kalscheur and Garcia, 2008). However, DDGS may result in a decrease of protein and AA availability due to possible protein damage from heat during the drying process (Kleinschmit et al., 2007; Kalscheur and Garcia, 2008). Wet distiller's grain with soluble is often lower in price on a DM basis compared to DDGS; however, producers must have proper facilities to store and handle WDGS. Methods to store and handle WDGS are challenges producers face (Kalscheur and Garcia, 2008). Wet distiller's grain with soluble has other advantages aside from cost which include higher palatability and usage as a ration conditioner to dry diets with smaller particle sizes. Total mixed rations (**TMR**) that contain 10-20% WDGS maintain greater homogeneity as dry particles stick together which results in less particle separation and less sorting by cattle. Wet distiller's grain with soluble can be partially dried to bring the DM up to between 45-55%. This product is called modified wet distiller's grain with soluble (**MWDGS**). Nutrient composition is usually similar to that of WDGS and DDGS (Kalscheur and Garcia, 2008).

Effects on manure

The increase in nutrient concentrations in WDGS or DDGS has led to further challenges in managing excreta. Wet distiller's grain with soluble in feedlot diets increases N, P, and S contents relative to conventional feedlot diets. Elevated levels of CP and fats in DGS diets create challenges managing manure as well. Undigested organic residues such as proteins,

carbohydrates, and fats compose livestock excreta. Volatile organic compounds (**VOC**), ammonia (**NH_3**), volatile fatty acids (**VFA**), S- compounds, and aromatic compounds are formed by aerobic and anaerobic digestion of organic residues in manure by bacteria (Mackie et al., 1998; Spiehs and Varel, 2009). Volatile organic compounds present in livestock diets contribute largely to negative odor perceived by humans (Zahn et al., 1997; Powers et al., 1999; Zahn et al., 2001). Cattle fed diets with DGS ranging from 20 to 40% of the diet DM will have elevated levels of CP, fat, and minerals particularly P and S. These excess nutrients can potentially contribute to environmental pollution from increased N-emissions, H_2S emissions, P runoff, and greater odor production (Varel et al., 2008). Studies conducted by Koziel et al. (2006) reported that the odorant *p*-cresol cause much of the overall odor impact on swine and cattle operations. Cresol originates from amino acids tyrosine, tryptophan (Mackie et al., 1998), and phenylalanine (Mohammed et al., 2003). Distiller's grain with soluble is approximately three times higher in these 3 AA's as well as others due to the greater concentration of CP compared to traditional corn fed diets (NRC, 1998; Stein et al., 2006; Varel et al., 2008). Greater concentrations of other AA's such as methionine and cysteine along with increased concentrations of sulfates in diets high in either WDGS or DDGS lead to an increase in reduced S or sulfides that can potentially form elevated levels of emitted H_2S from the manure compared with conventional corn-base diets (Shurson et al., 1998; Varel et al., 2008).

ROLES OF SULFUR IN THE BODY

Importance of dietary S

Many macro and micro minerals are required by ruminal bacteria and the host ruminant animal. Sulfur is among these mineral requirements as it is a necessary component of amino acids cysteine and methionine (Kung, 2008), as well as the B-vitamins biotin and thiamine along with other organic compounds (Crawford, 2007). Adequate levels of S in ruminant diets are also essential for healthy rumen micro-flora and animal performance (Loneragan et al., 2001). Sulfur deficiency can lead to decreased microbial protein synthesis, decreased microbial population, decreased OM digestibility, and depressed lactate utilization (Whanger and Matrone, 1970; Rumsey, 1978). Cattle fed diets deficient in S often suffer anorexia, weight loss, signs of depression, lethargy, and death (Starks et al., 1953; Slyter et al., 1988; NRC, 1996). Concern about S consumption in the feed is becoming a more popular issue due to feeding high levels of DGS, which has nearly a 3-fold greater concentration of S compared to corn. Distiller's grain with soluble creates other challenges because of the variability of S and minerals found in the co-product between and within processing facilities.

Sulfate-reducing bacteria

The NRC (2005) reports that dietary S for ruminant animals should be between 0.18 and 0.24% of DM to support microbial growth; and to provide an adequate level of S-containing compounds for the host animal (Crawford, 2007). Ruminant animals are capable of utilizing both organic and inorganic forms of S due to sulfate-reducing bacteria (**SRB**) that exist within the rumen via anaerobic respiration (Liamleam and Annechhatre, 2007). Sulfate-reducing bacteria are capable of reducing inorganic S compounds, particularly sulfate ($\mathbf{SO_4^-}$) to sulfides ($\mathbf{HS^-}$, $\mathbf{S^{2-}}$, $\mathbf{S^\circ}$, or $\mathbf{HSO_3^-}$) as part of a process to form microbial protein or for absorption and oxidation to

sulfate within the liver (Anderson, 1956; Kandylis, 1983; Fron et al., 1990). Inorganic S can be found in the form of ammonium sulfate (**$(NH_4)_2SO_4$**), copper sulfate (**$CuSO_4$**), potassium sulfate (**K_2SO_4**), calcium sulfate (**$CaSO_4$**), and sodium sulfate (**Na_2SO_4**) along with others (Kung, 2008). The site of digestion for organic compounds synthesized by microbes usually takes place in the abomasum and small intestine. The organic compounds are absorbed for utilization by the host animal (Block et al., 1952; Anderson, 1956; Fron et al., 1990).

The SRB are grouped according to their different mechanisms used to reduce S to sulfides. The two different mechanisms used to reduce S are assimilatory processes or dissimilatory processes. Typically, a dissimilatory process is used for energy production, while an assimilatory process is used to reduce SO_4^- to form biological compounds necessary for cell survival (Odom and Singleton, 1993; Kung, 2008). Both forms of SRB exist within the rumen but dissimilatory groups are responsible for the reduction of SO_4^- to HS^- and S^{2-} to allow protonation of S to form H_2S. Many forms of bacteria are capable of forming sulfides; however, *Desulfovibrio* organisms are the main SRB in the rumen (Cumming et al., 1995; Kung, 2008). Studies from Cumming and others (1995) showed that SRB did not increase proportionally with the increase of S concentration in the diet. However, adaptation to high levels of S was noted after 10 to 12 d as ruminal organisms began to have a greater capacity to produce sulfides. The rate of sulfate reduction by ruminal bacteria increased with high dietary S according to studies conducted by Oliveira et al. (1997).

Hydrogen sulfide production in the rumen

The amount of S-containing compounds found inside the rumen will determine the extent of dissimilatory sulfate reduction from ruminal microbes (Kung, 2008). According to Odom and

Singleton (1993), the sulfide compounds that are predominantly formed in the dissimilatory process are S^{2-}, S°, HS^{-}, or HSO_3^{-}. These reduced forms of sulfide have a pKa value near 7 and are readily protonated inside a rumen that typically has a pH range of 5.5 to 7.2. This protonation contributes to most of the H_2S produced in the rumen. A small amount remains in the liquid phase in a variety of S-containing compounds (Kung, 2008). The increase of S in diets from feeding high levels of DGS not only contributes to a possible increase in H_2S production, but could negatively impact animal performance and health.

FEEDING HIGH SULFUR DIETS

Effects of high S diets on performance and health

Several studies suggest that high levels of dietary S lead to decreased feed intake and feed efficiency. Bouchard and Conrad (1974) reported lower DM dietary intake in dairy cows when S was included in the diet at concentrations of 0.35% or greater. Bolsen et al. (1973) reported a 32% reduction in feed intake when cattle were fed 0.41% S with high concentrate diets. A study from Rumsey (1978) showed similar results, as the addition of high S levels in feedlot diets decreased feed intake and weight of steers fed high concentrate diets. The addition of 0.5% S (as calcium sulfate or sodium sulfate) in lambs decreased growth rate, depressed feed intake, and adversely influenced feed conversion (Johnson et al., 1968; Kandylis, 1983). A more recent study from Zinn et al. (1997) reported cattle diets with moderately excessive levels of S (0.25% of DM) had detrimental effects on ADG, DMI, G:F ratio, dietary NE, and longissimus muscle area. In addition to decreasing animal performance; high dietary S can also lead to S-toxicity, polioencephalomalacia (**PEM**), decreased liver Cu stores (Smart et al., 1986), diarrhea, increased H_2S emissions, and death (Bird, 1972; Bulgin et al., 1996; Loneragan et al., 2001).

Sulfur toxicity

Ingestion of large amounts of S in ruminant animals can lead to acute S-toxicosis and even death. Although S can be tolerated at high levels in monogastrics, ruminal bacteria are capable of reducing S usually in the form of SO_4^- to S^{2-}, which is readily absorbed into the blood stream through the rumen wall and lungs. Once S is absorbed in the bloodstream, it can cause several metabolic and respiratory problems to the animal (Bray, 1969; Kung, 2008). During the process of eructation and inhalation, ruminant animals absorb H_2S gas in the lungs

where H_2S enters the blood stream (Bird, 1972; Gould, 1998). The immediate signs associated with S-toxicosis include thrashing, kicking at the stomach, staggering, and moaning followed by death within 48 h. This short time frame suggests that ruminal bacteria have a high capacity to produce sulfide without the need for adaptation (Kung, 2008). High levels of sulfide in ruminal gas have been reported to cause respiratory distress, decreased feed intake, and decreased ruminal motility (Bird, 1972; McAllister et al., 1992).

Oxidative metabolism and production of ATP are negatively affected by excess S through inhibition of: carbonic anhydrase, an enzyme that forms bicarbonate; dopa oxidase, which produces melanin; along with catalase and peroxidase enzymes that breakdown hydrogen peroxides (**H_2O_2**) to water (**H_2O**) and oxygen (**O_2**). Additionally, dehydrogenases and dipeptidases are affected, decreasing hydrolysis of AA in the GI tract (Short and Edwards, 1989). In addition, sulfides are also thought to block the enzyme cytochrome c oxidase, which can cause several metabolic disorders (Kung, 2008). Sulfides in the blood stream may also hinder oxygen transport to tissues by binding to hemoglobin creating sulfhemoglobin. Respiration may also be effected by sulfides through inhibition of normal sensory functions of the carotid body (chemoreceptors), which help regulate and maintain partial pressure of O_2 and carbon dioxide (**CO_2**) (Bulgin et al., 1996; Kung, 2008).

Polioencephalomalacia

Polioencephalomalacia is a disorder of the central nervous system that softens the grey matter of the brain in ruminant animals and is often associated with a deficiency in thiamine or consumption of plants containing high levels of thiaminase (Goonerate et al., 1989; Olkowski et al., 1992). Excess S has an antagonist relationship with thiamine, rendering thiamine biologically

unavailable to the animal and subsequently putting the ruminant animal at risk of PEM. Clinical symptoms attributed with PEM include blindness, head pressing, and circling. If PEM goes untreated; symptoms progress to lameness, convulsions, and death (Merck, 1991; Kung, 2008). Polioencephalomalacia can be easily determined by necropsy of the brain and heart. Thiamine is a cofactor of thiamine pyrophosphate (**TPP**) for the TCA cycle and the pentose shunt, and lesions will be present in the brain and heart when thiamine is deficient (Morck, 1993). Symptoms of PEM have been reported in cattle fed diets between 0.4 to 0.5% S (Gould et al., 1991), but in some cases cattle have been fed up to 1.5% S without signs of PEM or S-toxicosis (Slyter el al., 1986). This is evidence that a likely interaction does occur with S and other micronutrients within the rumen that might determine the effect and rate of S that becomes reduced and absorbed either through the rumen wall or lungs (Kung, 2008).

Risks associated with hydrogen sulfide emissions

High levels of sulfates and other forms of S in diets are reduced in the rumen by SRB to produce H_2S that are not only hazardous to the animal but can cause potential air quality issues by contributing to environmental concentration of H_2S gas (Powers et al., 2006). Excess generation of H_2S in the rumen will depress normal rumen function and could lead to respiratory problems (Kandylis, 1983), since nearly 60% of belched gas can be inhaled into the lungs (Bulgin et al., 1996). Hydrogen sulfide gas is also very dangerous for humans to inhale. Exposure to H_2S at concentrations between 200 to 500 ppm has resulted in sudden onset of hemorrhagic pulmonary edema that can be fatal in humans. At concentrations greater than 2000 ppm of H_2S, respiratory paralysis occurs after only 1 or 2 breaths and is followed by convulsions and death within minutes (Osweiler et al., 1985; Gerber et al., 1991). Even at lower

concentrations (50 to 200 ppm), H_2S could cause respiratory irritation in humans (Green et al., 1991). Long term exposure to low levels of H_2S can cause chronic respiratory problems and ocular irritation (Hays et al., 1972). Increased sulfides in the air and rain can be corrosive to structures, particularly to iron and steel (Odom and Singleton, 1993). Excess H_2S in ambient air can have a detrimental impact to the environment, such as acid rain and eutrophication (Guang-hui et al., 2006).

Manure pits beneath animal confinement facilities and slatted floor feedlots allow decomposition of manure, which is a main contributor to H_2S production. Hydrogen sulfide has a low solubility in water and therefore will mostly remain trapped in bubbles in the manure (Pickrell, 1991; Hooser et al., 2000). Agitation of the manure during removal can cause a rapid release of H_2S at lethal concentrations within and surrounding confinement facilities and/or feedlots (Osweiler et al., 1985; Donham et al., 1988). Increasing levels of protein or other S-containing organic matter in animal diets may lead to an increase of H_2S in the manure and a greater potential risk during manure handling and removal to both the humans and animals (Hooser et al., 2000). These potential dangers and implications associated with total reduced sulfur (**TRS**) have led to stricter regulations on confinement and feedlot operations to control and monitor concentrations of TRS (Koelsch et al., 2004).

METHODS TO MEASURE SULFUR AND HYDROGEN SULFIDE EMISSIONS

Portable field analyzers

Efforts to improve soil and air quality along with industry concern to regulate concentrations of dietary S have influenced the development and methods for measuring S. This allows better diet formulation for ruminant animals along with offering better strategies for proper waste management to decrease environmental effects. Measuring S and sulfides can be difficult due to the volatile nature of S-containing compounds, particularly H_2S (Shurson et al., 1999). Reduced forms of S are very reactive in the air which causes a rapid inter-conversion of various S forms (Spoelstra, 1980). Methods to measure sulfates and sulfide compounds were originally developed for soil tests. Gravimetric and turbidimetric procedures are common methods used to measure sulfates in wastewater (Sawyer and McCarty, 1978). Methods to measure sulfides include colorimetric or volumetric methods. Both methods pose limitations in separating inorganic from organic S, as well as differentiating elemental S from sulfate to determine percentage of sulfide (Shurson et al., 1999). A study was conducted at the University of Nebraska to determine H_2S concentrations near beef cattle feedlots. The study used two Jerome 631-S analyzers with memory to survey TRS concentrations at 15-minute intervals with the analyzers placed approximately one meter from the ground surface (Koelsch et al., 2004). The placement of analyzers was important because H_2S gas is heavier than air and tends to be at highest concentrations near the surface of manure storage (Shurson et al., 1999). Jerome 631 analyzers were developed by Arizona Instrument Corporation, Jerome Instrument Division (Tchobanoglous and Burton, 1991). Similar portable H_2S analyzer by ASTM (1996) can

continuously measure H_2S as low as 1 ppb by measuring the rate of change of reflectance. The cost associated with these instruments may limit their use in the field.

Ruminal gas cap

Sulfate-reducing bacteria in the rumen convert S compounds into H_2S, which accumulates in the area between the surface of ruminal fluid and the ruminal wall. Ruminal gas caps have been used extensively as a way to measure H_2S concentrations within the rumen of cattle. A common method for the gas cap procedure is to make an incision on the left paralumber fossa. An 18 gauge 89 mm spinal needle is inserted through the prepared body wall into the ruminal gas cap. The needle is then attached to a calibrated H_2S detector tube. A volumetric gas sampling pump draws the gas from the ruminal gas cap into the detector; usually 50-300 ml of gas is collected to obtain H_2S readings (Gould et al., 1997 and Loneragan et al., 1998). Ruminal gas caps are effective in determining enteric H_2S production within the rumen but does not account for gas produced further down the GI tract or from excreta. Another disadvantage associated with using ruminal gas caps is several gases cannot be recorded at a time, making it difficult to determine total emissions. Vannes et al. (2009) conducted a study at University Nebraska to measure H_2S levels post feeding. The study used cannulated cattle and a gas collection device was inserted directly inside the rumen of each steer. Hydrogen sulfide levels within the rumen were then determined by pulling samples from the gas collection device and performing an in vitro assay to measure sulfide.

In vitro sulfide determination

Acidic conversion of sulfide to methylene blue is very specific and potentially the most sensitive assay available to accurately measure H_2S. This method is conducted by trapping H_2S gas in water with a pH 8 or greater followed by the addition to 2 reagents, first the addition of 7.2 N hydrochloric acid (**HCl**) with 0.02 M *N*,*N*-dimethyl-*p*-phenylenediamine (**DPD**) then the addition of 1.2 N HCl with 0.03 M ferric chloride is immediately followed. Samples are quickly vortexed and placed in the dark to allow proper formation of methylene blue (**MB**). Greater concentrations of H_2S in solution will produce a darker blue color. Samples can then be plated and ran through a spectrophotometer with a wavelength near 650 nm to determine absorption. Absorption levels are then compared to a standard curve to determine concentration of H_2S in each solution (Siegel, 1964). Methylene blue color is formed as the DPD reagent is oxidized by iron ($\mathbf{Fe^{3+}}$), which reacts with H_2S (Moneras et al., 2010).

Animal air chambers

Recent research has been conducted by housing livestock animals in open circuit respirometers. Much of the data currently has been reported from studies at Iowa State University and Michigan State University. The Animal Air Quality Research Facility (**AAQRF**) at Michigan State University measures incoming or ambient air gas concentrations of O_2, CO_2, methane ($\mathbf{CH_4}$), non-methane total hydrocarbons (**NMTHC**), H_2S, sulfur dioxide ($\mathbf{SO_2}$), ammonia ($\mathbf{NH_3}$), nitrous oxide ($\mathbf{N_2O}$), and nitrogen oxides (**NOx**; NO_2 and NO^-). Expelled air from animal chamber rooms is then adjusted for incoming air gas concentrations, temperature (C°), and air-flow (m^3/min). Sulfide containing gas concentrations are measured using a TEI

450i Pulsed Fluorescence SO_2-H_2S-CS Analyzer (Thermo Fisher Scientific, Franklin, MA, USA). The 450i analyzer operates by H_2S converting to SO_2 by exciting molecules under different wavelengths using ultraviolet (**UV**) light (Thermo Fisher Scientific, Franklin, MA, USA). Ammonia and NOx gas concentrations were measured using a TEI 17C Chemiluminescence Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) while N_2O is measured using an Innova 1412 Photoacoustic Field Gas Monitor. Methane and NMTHC gas concentrations were measured using a TEI 55C Gas Chromatographic Analyzer and O_2 and CO_2 were measured using a BINOS 100 2M Dual-Channel Infrared Gas Analyzer.

STRATEGIES TO MITIGATE HYDROGEN SULFIDE PRODUCTION

Biocides

Biocides such as hypochlorite (Odom and Singleton, 1993), methylenebis thiocyanate (Zou and King, 1995), and gentamicin (Tanimoto et al., 1989) have been used in industrial situations to control sulfide production. Although biocides may be able to limit SRB and reduce production of S-containing emissions, they are not recommended for use in ruminant diets because of their broad anti-microbial spectrum which can negatively impact ruminal fermentation and can be highly toxic to the animal (Kung, 2008).

Ionophores

Ionophores have been considered as a strategy to manipulate production of desirable end products such as VFAs and microbial proteins and minimize production of undesirable greenhouse gases (Kung, 2008), such as CH_4, CO_2, and N_2O. Rumensin^{TM} (monensin) is commonly used as a feed additive in ruminant diets to improve feed efficiency. Monensin is an ionophore capable of altering ruminal fermentation by inhibition of hydrogen-producing bacteria that have a gram-positive cell wall structure. Antibiotics and ionophores are typically administered for resistance against pathogenic gram-positive bacteria (Nagaraja, 1995). Sulfate-reducing bacteria are gram-negative, so ionophores would not have a direct effect on inhibiting sulfide production. Indirect effects are thought to occur when monensin and other ionophores are fed. A possible example would be that monensin selects against hydrogen and formate producing bacteria that could result in the decrease of CH_4 and H_2S production (Kung, 2008).

Consequently, more propionate and less acetate and butyrate are produced by selecting against formate producing bacteria (Van Nevel and Demeyer, 1996). Propionate is a VFA that is a product commonly found in ruminants fed high concentrate diets and yields 34 ATP/mol of

glucose. Acetate and butyrate are other major VFA's that are end products of digestion in ruminants but only yield 20 and 25 ATP/mol of glucose, respectively.

Both methanogens and SRB are dependent on hydrogen as a substrate to form CH_4 and H_2S, repectively. By inhibiting production of hydrogen, methanogens and SRB would be depleted, so theoretically ionophores would decrease CH_4 and H_2S emissions (Van Nevel and Demeyer, 1996). An in vitro study conducted by Quinn et al. (2009) indicated that the inclusion of 3 different ionophores (lasalocid sodium and monensin sodium at 5 mg/L or laidlomycin propionate at 1.65 mg/L) and 2 antibiotics (chlortetracycline hydrochloride at 5 mg/L and tylosin tartarate at 1.25 mg/L) in the presence of S had similar acetate, propionate, and acetate:propionate as the control culture. In addition, when S was approximately 0.42% of substrate DM, the 3 ionophores and 2 antibiotics did not affect production of H_2S gas in an in vitro rumen culture system. Even if the inclusion of ionophores did increase propionate and decreasing greenhouse gas (**GHG**) production, there is concern that adaptation may occur in the ruminal microbiota and inhibition of H-dependent bacteria may be deminished over time (Van Nevel and Demeyer, 1996). More studies should be conducted to determinine if ionophores and antibiotics are effective at enhancing greater propionate production and decreasing CH_4 and H_2S emissions.

Effect of feeding urea on S and N balance

Protein rich feeds are major contributors to S concentration in ruminant diets. One consideration to decrease H_2S emissions is to feed a ration with decreased levels of dietary S while maintaining high levels of N within the diet. The use of urea as a non-protein N (**NPN**)

supplement in ruminant rations can dramatically reduce dietary S. In many rations where urea is fed, supplementation of S may be required to meet dietary requirements. Sulfur deficiencies can limit NPN utilization in purified diets but supplementation with inorganic sulfate can negate issues associated with S deficiencies (Thomas et al., 1951). In order to feed urea as a source of NPN and to lower dietary S as a strategy to decrease H_2S emissions, adequate S and N balance must be met to maximize utilization. Elemental S has been used in ruminant diets deficient in S but is poorly utilized compared to Na_2SO_4 and methionine (Spais et al., 1968; Johnson et al., 1971). Some forms of supplemental methionine (methionine hydroxyl) have shown negative effects on DMI and milk protein production in dairy cattle (Goodrich and Tillman, 1966; Bray and Hemsley, 1969; Rosser et al., 1971; Salsbury and Chandler, 1971). A study conducted by Starks et al. (1953) suggested that lambs retained more N and S when elemental S was added to diets supplemented with urea. The study compared 2 homo-nitrogenous diets at 2.46 and 2.47% of dietary DM and two S levels (0.062% versus 0.705% of DMI).

Studies on urea with S and N balance are important on establishing that a relationship between N and S does exist for retention rates and to maximize utilization of NPN. However, in the case of feeding DGS where high concentrations of CP and S are present, feeding urea in most cases would not be practical because high levels of N would be present in moderate to high levels of DGS diets. To utilize DGS, other strategies should be considered to decrease H_2S emissions.

Sulfur, molybdenum, and copper compound

Molybdate (**MoO_4**) has been identified as a compound that is capable of inhibiting SRB. It is thought that MoO_4 works as an analog of sulfate, blocking the sulfate activation step that is

catalyzed by ATP sulfurylase (Oremland and Capone, 1988). Conflicting research has been reported on whether MoO_4 specifically inhibits SRB or if it has inhibitory affects on other ruminal bacteria as well. Jones et al., (1982) concluded that methanogenesis is inhibited by inclusion of MoO_4. Whereas a study from Westerman and Ahring (1987) showed that low levels of MoO_4 (1 mM) actually increased CH_4 production. A study from Kung (2008) demonstrated MoO_4 was a specific inhibitor of SRB and did not have any effect on CH_4 or hydrogen production. Sodium molybdate decreased H_2S concentrations in ruminal gas caps with cattle fed high S diets, however results were not consistent among cattle (Loneragan et al., 1998). In addition, the study conducted by Loneragan et al. (1998) demonstrated that inclusion of MoO_4 dramatically decreased liver Cu stores in cattle.

Molybdenum (**Mo**) and S react to form tetrathiomolybdates that then react with copper (**Cu**) and particulate matter in the rumen, forming highly stable compounds that cannot be digested and absorbed (Allen and Gawthorne, 1987; Suttle, 1991). When Mo intake exceeds 1 mg/kg of DMI, a reduction in Cu absorption in ruminant animals usually results (Suttle and Field, 1983; Suttle et al., 1984). The synergetic effect between Mo and S begins with the substitution of S for oxygen in the MoO_4^{2-} ion to ultimately yield tetrathiomolybdate (MoS_4^{2-}).

$$MoO_4^{2-} \rightarrow MoO_3S^{2-} \rightarrow MoO_2S_2^{2-} \rightarrow MoOS_3^{2-} \rightarrow MoS_4^{2-}$$

Tetrothiomolybdate has the potential to bind ruminal Cu ions to form MoS_4Cu, rendering the entire complex biologically unavailable to the animal. The compound formed between S, Mo,

and Cu molecules in plasma is highly stable and usually is a result of excess Mo in the diet, exceeding 10 mg/kg of DMI (Mills et al., 1978; Mills, 1980).

Molybdenum toxicity and Cu deficiency are often areas of concern when the MoS_4Cu compound forms. Symptoms that are often expressed by Mo toxicity include scouring, achromotrichia, anemia, and weight loss (Lesperance et al, 1985). Lesperance et al. (1985) found that feeding Mo as high as 100 ppm in the diet led to high retention rates of Mo (105 mg/day) and elevated plasma Cu levels. The authors recommended plasma and urinary Mo were both effective indicators of Mo intake. Another study from Marcilese et al. (1970) also indicated feeding Mo increased Cu excretion in the urine of sheep. The increase in Cu excretion is thought to be caused by thiomolybdates binding to albumin to cause a conformational change in the protein, and thus increases the affinity of albumin for Cu (Woods and Mason, 1987). Increasing levels of Mo in ruminant diets requires an increase in dietary Cu to prevent symptoms of molybdenosis. Ingestion of high levels of Mo over time may also cause hypocupremia (Underwood, 1977). Because of the interrelationship between S, Mo, and Cu; supplemental Mo and Cu may serve as a strategy to prevent reduction of dietary S and therefore restrict production of H_2S gas in ruminant animals.

Iron binding effects with sulfides

Iron (**Fe**) may inhibit Cu reserves in ruminant diets at inclusion levels as low as 250 mg Fe/kg of DMI (Mason, 1979). Also, Fe^{2+} may bind S^{2-} to form an insoluble ferrous sulfide (**FeS**) complex. Although not well understood, it is thought the S^{2-} is donated by the Cu binding transport proteins in the mucosa which are rich in sulfhydryl groups. With the loss of S^{2-}, the formation of the mercaptide bonds are blocked, which are necessary for binding Cu ions (Mason,

1979). Ferrous sulfide complex mechanism is not well understood, but it is thought that FeS is formed in the rumen and becomes soluble in the abomasum where the sulfide may dissociate and form insoluble complexes with Cu (Gangelback et al., 1994; Suttle et al., 1984).

Sulfide inhibition by 9,10 anthraquinone

Another compound- 9,10 anthraquinone (AQ) has been reported to decrease CH_4 production in laboratory in vitro studies (Garcia-Lopez et al., 1996) and inhibit reduction of sulfate in the rumen (Hession et al., 1995; Kung et al., 1996; Bracht and Kung, 1997; Kung et al., 1998). Anthraquinone decreased sulfide production in a diet containing 1.09% S to levels below that found in a diet with only 0.29% S (Kung, 2008). Inhibition of sulfide production from AQ is caused by possible uncoupling of the electron transport chain due to the redox potential of anthraquinones. Without sufficient energy in the form of ATP, SRB cannot continue to reduce ruminal S to produce sulfides. By restricting production of sulfides, H_2S formation would be limited (Cooling et al., 1996; Kung, 2008).

REVIEW OF RUMINANT GAS EMISSIONS

Little research has been conducted in animal air chamber rooms with feedlot cattle fed high levels of S and the effects on H_2S emissions. Furthermore, limited research is available on strategies to mitigate H_2S emissions in ruminant animals using in vivo forms of measurement. Feedlot rations with high levels of DGS typically have high concentrations of S. Increased demand for ethanol and growing numbers of ethanol plants in the USA has provided greater production and availability of DGS for implementation in animal diets. Although DGS may serve as an economical feed alternative in place of corn and SBM, feeding DGS does present challenges due to the variability of the feed and the high dietary mineral concentrations. Among these challenges, ruminant animals are often put at risk for possible health issues related to mineral toxicities associated with DGS diets. In addition, feeding DGS increases environment risks with manure handling, GHG, and other hazardous emissions.

Continued research is vital to better understand effects DGS may have on emissions in ruminant animals. The use of animal air chambers to measure emissions could offer new information in the area of studying in vivo fermentation and gas emissions from ruminant animals. Additionally, use of mineral compounds may be the most advantageous method to decrease emissions while limiting negative effects on ruminal fermentation and animal health.

CHAPTER 2: AN INTRODUCTION AND OBJECTIVE STATEMENT

INTRODUCTION

The rapid growth in the ethanol industry over the last decade has led to a surplus of distiller's grain with soluble (**DGS**). Ethanol production is currently the second largest component of corn demand in the U.S., accounting for 30% of the total gross corn utilization during the 2008/09 marketing season (Renewable Fuels Association, 2010). Feeding DGS has become a popular energy and protein replacement relative to corn and soybean meal in livestock diets. According to the USDA Livestock & Grain Market News (July 15, 2011), dried distiller's grain with soluble (**DDGS**) has consistently offered a favorable price compared to corn over the last 2 years. The increased availability and reasonable price has encouraged feedlot managers to increase the levels of DGS in cattle diets. However, high inclusion levels of DGS can have negative effects on animal health and the environment. During the dry milling process for production of ethanol, starch is removed from the corn kernel, leaving fat, crude protein (**CP**), fiber, and other minerals such as sulfur (**S**) and phosphorus (**P**) 3-fold more concentrated within DGS compared to corn (Klopfenstein et al., 2008). Additionally, sulfuric acid is added during the fermentation process as a cleaning agent and to regulate pH. This further contributes to the increase of S in DGS (Vannes et al., 2009; Kelzer et al., 2010). Buckner et al. (2008) reported S concentrations in DGS varying from 0.44% to as high as 1.5%. This increase in mineral concentrations in DGS can compromise the animal's health, create challenges in waste management, as well as increase the concentrations of hazardous gas emissions from manure storage. Part of the risk associated with feeding S to ruminant animals is the production of H_2S in the rumen from sulfate-reducing bacteria. Loneragan et al. (1998) reported H_2S concentrations in the ruminal gas cap as high as 18.77 mg/L or 13,500 ppm in cattle fed high S diets. Ruminal

H_2S is inhaled into the lungs where it is absorbed and enters the bloodstream (NRC, 2005; Crawford, 2007). It has been reported that 60% of the eructated H_2S is absorbed in the lungs (Bulgin et al., 1996). Therefore, cattle producing high concentrations of ruminal H_2S from high S diets should eructate approximately 40% of the enteric H_2S produced into the environment.

Studies have reported that molybdate (MoO_4) is capable of inhibiting sulfate-reducing bacteria and decreasing H_2S production in ruminal gas caps (Oremland and Capone, 1988; Loneragan et al., 1998; Kung, 2008). Supplemental MoO_4 may also inhibit methanogens, resulting in a decrease of enteric CH_4 production (Jones et al., 1982). Additionally, Molybdenum (**Mo**) and S react to form tetrathiomolybdates that then react with copper (**Cu**) and particulate matter in the rumen, forming highly stable compounds that cannot be digested and absorbed (Allen and Gawthorne, 1987; Suttle, 1991). Because of the interrelationship between S, Mo, and Cu; supplemental Mo and Cu may serve as a strategy to prevent reduction of dietary S and therefore restrict production of H_2S gas in ruminant animals.

Cattle diets with increased levels of DGS also offer elevated levels of nitrogen (**N**), which may contribute to greater excreted N and emitted nitrogenous gases from the manure (Mackie et al., 1998; Spiehs and Varel, 2009). Nitrogenous gases produce strong odors and can cause acid rain from the elevated levels of environmental NH_3 and NOx emissions, as well as contribute to greenhouse gas (**GHG**) emissions by producing N_2O gas (Pollution Prevention and Abatement Handbook, 1998; Intergovernmental Panel on Climate Change, 2007).

OBJECTIVE STATEMENT

The objective of trial 1 was to determine what affects DDGS fed at 0, 40, and 60% of the dietary DM would have on gas emissions generated from growing steers. We hypothesized that S- and N-emissions would be greater as the level of DDGS increased in the cattle diet. Based on other reports, we expect the steers to emit H_2S gas from ruminal fermentation. The objective for trial 2 was to determine whether supplementation of 8 ppm Mo and 90 ppm Cu into a 40% DDGS diet would decrease H_2S emissions from growing steers as well as determine how Mo and Cu may affect other emissions. The use of Mo and Cu as a dietary treatment at 8 and 90 ppm, respectively, was hypothesized to mitigate H_2S and possibly CH_4 gas by either inhibitory effects or by forming an insoluble compounds (MoS_4Cu) in the rumen and manure. Lastly, the third objective was to determine the relative amounts of gas emissions that originates directly from the animal (ruminal and enteric) versus short-term manure storage.

CHAPTER 3: LEVELS OF DISTILLER'S GRAIN WITH SOLUBLE AND STRATEGIES TO MITIGATE SULFUR-CONTAINING EMISSIONS

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SUMMARY

A rising concern with feeding high levels of DGS is its high S content and the effects it might have on S-containing emissions from gas produced in the rumen and excreted feces. Two trials were conducted with 12 Holstein steers randomly assigned individual environmentally-controlled rooms to monitor gas production. In trial 1, steers (3 treatments, 4 steers/treatment) were assigned to diets containing either 0 (control), 40, or 60% DDGS. In trial 2, treatments were the same except the 60% DDGS dietary treatment was replaced with a 40% DDGS diet as in trial 1 fortified with 8 ppm Mo and 90 ppm Cu (40% DDGS+) to potentially mitigate S-containing gas emissions. Fecal bags were placed on steers at the end of each trial to determine the effects of separating urine and feces on gas emissions. In trial 1, feeding DDGS increased H_2S mass output ($P = 0.05$) and output adjusted for DMI ($P = 0.04$) compared to emissions produced from the control diet. In trial 2, the 40% DDGS+ diet tended to decrease H_2S emissions when adjusted for S-intake ($P = 0.08$). In both trials, placement of fecal bags to separate urine and feces nearly abolished H_2S emissions ($P < 0.01$), indicating that H_2S gas was not directly emitted from enteric ruminal fermentation in the animal. Supplementing Mo and Cu in cattle diets high in S may be an effective strategy to decrease H_2S emissions generated by cattle excreta.

Key words: distiller's grain with soluble, hydrogen sulfide, sulfur dioxide, molybdenum, copper

INTRODUCTION

Growth in the ethanol industry has increased the availability of distiller's grain with soluble (**DGS**). The increased availability and reasonable price have encouraged feedlot managers to include more DGS in cattle diets. During the dry milling process used to produce ethanol and DGS, starch is removed from the corn kernel, leaving fat, crude protein (**CP**), fiber, and other minerals such as sulfur (**S**) and phosphorus (**P**) nearly three times more concentrated within DGS compared to corn (Klopfenstein et al., 2008). Sulfuric acid is added during the fermentation process as a cleaning agent and to regulate pH, this increases the amount of S in DGS as well (Vannes et al., 2009; Kelzer et al., 2010). Buckner et al. (2008) reported S concentrations in DGS as high as 1.5%. High levels of S can compromise animal health and increase the risk of S toxicity or Polioencephalomalacia (**PEM**). These conditions can severely depress animal performance, and if untreated, even cause death. Another potential concern with feeding higher levels of DGS is environmental pollution from increased hydrogen sulfide (**H_2S**) emissions (Varel et al., 2008). One possible strategy to mitigate H_2S emissions is to add supplemental copper (**Cu**) and molybdenum (**Mo**) to the diet to form a biologically unavailable compound between Cu-Mo-S before dietary sulfates (**SO_4**) can be reduced to H_2S within the rumen.

Molybdenum and S react to form tetrathiomolybdates (**MoS_4^{2-}**) or (**TM**) that then react with Cu and particulate matter in the rumen, forming a highly stable compound that is very difficult for the animal to digest and absorb (Gould et al., 2002). The formation of this compound is hypothesized to bind dietary sulfates before sulfate-reducing bacteria (**SRB**) within the rumen can reduce the sulfate to H_2S gas (Allen and Gawthorne, 1987; Suttle, 1991). However, feeding

excess Mo (>10 ppm) may put the animal at risk of Mo toxicity as well as Cu deficiency (Mills et al., 1978; Mills, 1980).

In trial 1, cattle were fed dry distiller's grain with soluble (**DDGS**) at levels of 0, 40, and 60% to determine the effects on S-containing emissions. Feeding high levels of DGS (dried or wet) in livestock diets increases the concentrations of CP, S, and P in the diet. Increased S concentrations cause an increase of H_2S production within the ruminal gas cap (Lonergan et al., 1998) and in theory would cause an increase of H_2S emissions from the animal by eructation or flatulation. In trial 2, cattle were fed diets containing levels of DDGS at 0, 40 and 40% with the inclusion of 8 ppm Mo and 90 ppm Cu. Supplementing additional Mo and Cu should reduce available dietary S and thereby mitigate H_2S emissions. The purpose of both trials was to determine if increased concentrations of dietary S through increased inclusion levels of DDGS would cause increased emission of H_2S gas and sulfur dioxide (SO_2) gas, and to determine the potential for supplemental Cu and Mo to reduce S-containing emissions.

MATERIALS AND METHODS

Gas collection

Two studies were conducted at Michigan State University at the Animal Air Quality Research Facility (**AAQRF**) to address concerns associated with high inclusion levels of distiller's grain with soluble. Approval for this study was provided by the Michigan State University Animal Care and Use Committee (AUF # 07/09-110-00). Individual Environmentally-controlled rooms at AAQRF monitor incoming and outgoing air from the 12 rooms for concentrations of oxygen (**O_2**), carbon dioxide (**CO_2**), methane (**CH_4**), non-methane total hydrocarbons (**NMTHC**), H_2S, sulfur dioxide (**SO_2**), ammonia (**NH_3**), nitrous oxide (**N_2O**), and nitrogen oxides (**NOx**; NO_2 plus NO^-). Before arrival to AAQRF, steers were held at Michigan State University's Beef Cattle Teaching and Research Center (**BCTRC**) where they were weighed and vaccinated for prevention of clostridial infections using Ultrabac-7 (Pfizer, New York, NY) and respiratory infection with Bovi-Shield GOLD® 5 (Pfizer, New York, NY).

At trial initiation; a two week adjustment time was allotted before steers were transported to AAQRF. During this time, steers (n = 12) were started on a corn-based concentrate diet (control diet). After the first week, steers (n = 8) were randomly adjusted to a 40% DDGS diet. The control diet consisted of 81% high moisture corn (**HMC**), 10% corn silage (**CS**), 5% soybean meal (**SBM**), and 4% mineral supplement. The 40% DDGS diet consisted of 40% DDGS, 46% HMC, 10% CS, and 4% mineral supplement. Steers were halter trained to lead for safe handling and to limit stress on the animal during the study at AAQRF. Four days prior to arrival at AAQRF, steers were housed in metabolism stalls at BCTRC to adapt them to living conditions that were similar to the individual, environmentally-controlled rooms at AAQRF. Steer weights

were taken prior to feeding the last 2 d before the steers were transported to AAQRF, animal weights were taken post-study as well. After 10 d of being housed in individual environmentally-controlled rooms during trial 1, 4 steers were randomly switched from the 40% DDGS diet to a 60% DDGS diet; establishing the 3 dietary treatments containing 0, 40, and 60% DDGS.

In both trials, 12 Holstein steers were placed into individual environmentally-controlled rooms with four steers per dietary treatment. The dietary treatments for trials 1 and 2 are shown in Table 3.1. Three levels of DGS (0, 40, and 60%) replaced corn in a finish diet in trial 1. All diets in trial 1 were top dressed with thiamine (200 mg/d) for each animal as a preventive step against PEM. Daily records were kept on eye and ear twitch count over 1 min during feeding shift to monitor physical symptoms associated with PEM. Visual symptoms of PEM were not detected in either trial 1 or trial 2. On d 5 of trial 1, a steer on the control diet had to be pulled off the study due to poor feed intake and formation of hematoma near the left hook bone. In trial 2, two levels of distiller's grain with soluble were fed (0 and 40%). A third treatment was identical to the 40% distiller's grain with soluble but it was fortified with 90 ppm Cu and 8 ppm Mo. The latter treatment was called 40% DDGS+. The source of Mo was sodium molybdate ($\mathbf{Na_2MoO_4}$) and the Cu source was copper chloride ($\mathbf{CuCl_2}$). Copper chloride was selected to minimize the amount of additional S in the diet.

Table 3.1 Dietary ingredients and composition for trial 1 and 2

	Trial 1			Trial 2		
Ingredient, % of DM	Control	40	60	Control	40	40+[2]
DDGS[1]	-	40	60	-	40	60
High moisture corn	81	46	26	81	46	26
Corn silage	10	10	10	10	10	10
Soybean meal	5	-	-	5	-	-
DGS supplement	-	-	-	-	4	4
BFS50 supplement	4	4	4	4	-	-
Total	100	100	100	100	100	100
			Diet composition			
Dry matter, %	67.7	74.8	76.0	68.6	73.6	74.5
			% of DM			
Ash	5.6	7.4	8.0	4.4	6.4	7.1
ADF	5.9	7.1	8.8	4.2	7.2	7.0
NDF	11.8	19.0	23.2	11.7	19.9	19.6
Starch	60.3	33.5	22.1	58.6	33.5	33.3
Ether extract	2.9	4.6	5.5	2.2	3.9	3.9
Crude protein	12.9	21.5	24.6	12.9	19.1	18.8
ADIP[3]	1.5	5.5	6.6	1.3	4.5	5.0
Gross energy, Mcal/kg	4.0	4.3	4.4	4.4	4.7	4.7

Ingredient, % of DM	BFS50 supplement	DGS supplement
Akey TM premix # 4™*	1.4	2.4
Limestone	24.9	71.5
Soybean meal, 48% N	48.3	-
Rumensin™ 80	0.3	0.4
TM salt	9.6	18.0
Vitamin E, 5%	0.2	0.1
Urea, 45% N	9.6	7.6
Potassium chloride	5.1	-
Selenium 90	0.7	-
Total	100	100

[1] DDGS- dry distiller's grain with soluble

[2] 40% + is fortified with supplemental 8 ppm molybdenum and 90 ppm copper

[3] Acid detergent insoluble proteins (ADIP) represent a portion of undegradable intake protein (UIP) that is completely indigestible to the cattle

* Akey TM premix # 4 composition: 9% Mg, 4% S, 0.02% Co, 1% Cu, 0.09% I, 2% Fe, 4% Mn, 0.03% Se, 4% Zn, 4,400,000 IU vitamin A, 550,000 IU vitamin D, and 5,500 IU vitamin E/kg (Akey Inc., Lewisburg, OH)

An adaption period was provided for cattle assigned to the control and 40% DDGS diets prior to their entry in the environmentally-controlled rooms. Steers assigned to the 60% DDGS diet were adjusted from 40 to 60% DDGS during the first 10 d in the chamber during trial 1. Cattle were allocated 1 d to adapt to the chamber rooms during trial 2. After the adjustment period emissions were recorded over a 14 d period in trial 1 and 23 d period in trial 2, using the last 4 d to determine mean emissions within treatment for both trials, which will be referred to as phase 1. The last 4 d were used to represent mean emissions at or near a steady state. Temperature was maintained near 16° C and air flow was closely regulated within a range of 17 m^3/min or 40 air changes per hour (**ACH**) while gaseous emissions were sampled throughout each d. Humidity in each chamber room was recorded but was not regulated. Each chamber, including ambient air recordings, were sampled for 15 min with the first 9.5 min used as a purge period, leaving the last 5.5 min of sampling as the recorded data. Sampling cycle of the 12 rooms plus the background air would take 3 h 15 min which allowed for 7 to 8 observations per room per d. Exhausted air was sampled within an aluminum duct in each room where Teflon coated sample lines would draw air to the gas analyzers by positive static pressure from each individually-sealed chamber room. Each room was also fitted with an in-house manufactured type-J thermocouple, and a Campbell Scientific™ HMP45C (Logan, Utah, Campbell Scientific, Inc.) temperature and relative humidity probe. The gas analyzer used to measure S-containing emissions was a pulsed fluorescence TEI 450i analyzer (Thermo Fisher Scientific, Franklin, MA, USA) for H_2S, and SO_2. Pulsed fluorescence operates under the assumption that H_2S can be converted to SO_2. Sulfur dioxide molecules absorb the ultraviolet (**UV**) light from a source lamp within the analyzer. Ultraviolet light is pulsed to increase optical intensity and provide greater

UV energy. This allows for better detection of low concentrations of SO_2. Sulfur dioxide molecules decay to lower energy states, emitting UV light at different wavelengths (Thermo Fisher Scientific, Franklin, MA, USA). Gas sampling and data collection were all computer controlled by LabView 8.2.1 software and the FieldPoint system (National Instruments, Austin, TX, USA). Analyzers reported gas in units of ppm; data were transposed into units of mg/min (Table 3.2) as output emissions. The units were then adjusted to 24 h and expressed as mass unit output, output adjusted for S intake (**SI**), and output adjusted for dry matter intake (**DMI**).

Table 3.2 Gas unit conversion chart

A.	Air Flow @ STP	1. STP	(((temp,° C) + 273° K) × 0.967 atm)
		2. m^3/min	STP × air flow, m^3/min
		3. L/min	(Air flow @ STP, m^3/min × 1000 L/ m^3)
B.	Emitted gas	1. ppm	Expired, ppm – incoming, ppm = emitted, ppm
		2. Units cancel	Emitted gas, mg/kg × 0.000001 kg/mg
C.	Volume of ideal gas @ 0°C & 760 mm Hg	1. mg/L	MW, g/mol/(22.414 L/mol) ×1000 mg/g
Adjusted equation for all gases		mg/min	(A.3) × (B.2) × (C.1)

Feed, feces and urine collection

During each gas collection period for trial 1 and trial 2 (phase 1), daily samples of total mixed ration (TMR) and feed weigh-back (orts) from each steer were collected and immediately placed in a freezer for storage. Orts were monitored daily to adjust the amount of TMR offered to 110% of the previous day's consumption. All feed samples were later freeze-dried and ground through a Wiley Mill (Thomas Scientific) to a 1 mm particle size or smaller. A manure pan was positioned behind each stall, and the stalls were set at a small slope to allow urine to flow back for collection of urine and feces in the manure pan. Once feces and urine mixture exceeded a 5 cm depth inside the pan, manure was mixed and partial removal was performed to maintain a minimum 5 cm depth. The removed excreta were weighed to calculate total excretion. An approximate 0.5 kg sample of manure mixture was collected during each removal and immediately stored in a freezer for later analysis. Excreta removal was conducted during the morning shift, whereas feeding along with TMR and ort collections were conducted in the afternoon. During each shift, chamber rooms and stalls were thoroughly cleaned and chamber rooms were only entered if they were 5 chambers or more ahead of the chamber room being sampled for emissions.

Feces and urine segregation using fecal bags

On d 15 of trial 1 and d 24 of trial 2, all manure and urine was removed and weighed from each fecal pan. Fecal bags were placed on each steer and clean pans were put back in place to collect urine only. Fecal and urine segregation took place during the last 3 d of trial 1 and the last 6 d of trial 2. In trial 1, only the last 2 d were used to record mean emissions and the last 4 d were used in trial 2. This duration of each trial will be referred to as phase 2. Urine pans were pulled twice daily and filtered through a screen before urine weight and volume were recorded.

Two 1% aliquots were collected of the composited daily urine collected, one aliquot was treated with 6 N HCl until urine pH reached 4 determined by a litmus paper test and the second sample was left untreated. All urine samples were stored in a freezer for later analysis. Fecal bags were removed from each steer and feces was weighed daily during the evening feeding. Mixed fecal sub-samples were collected at 5% of daily fecal weight and immediately placed in a freezer. Total mixed ration and orts samples continued to be collected during phase 2 and emissions were recorded for later comparison with phase 1 emissions.

Collections and mineral analysis

Feed samples including TMR and orts were freeze-dried and ground through a 1 mm screen in a Wiley Mill (Thomas Scientific) prior to mineral analysis. All samples excluding urine samples were prepared in a microwave digestion system by adding 10 ml of nitric acid to 0.5g feed and fecal samples. Samples in the 10 ml of nitric acid were covered in Saran^{TM} wrap and left in a ventilated hood over night. The samples were then transported in a pressurized Teflon-lined digestion vessel and placed in the microwave digester under 1200W at 100% power with a 30 min ramp time, max PSI of 180, at 190˚C for a 10 min hold time (Gengelback et al, 1994). Post digestion vessels were allowed time to cool for 5 min and then 2 ml of 30% hydrogen peroxide was added to each vessel and let sit unsealed for 15-30 min to allow more time to cool down for handling. Each digested sample was then poured into a separate 25 ml volumetric flask and vessels were rinsed with ddH_2O. The rinsed water was then added to the volumetric flask and additional ddH_2O was included to bring the total volume within the volumetric flasks to 25 ml (CEM Corporation, 1999). Each 200 µl digest and urine sample were pipetted and diluted with 5 ml of a solution containing 0.5% EDTA and Triton X-100, 1% ammonia hydroxide, 2%

propanol and 20 ppb of Sc, Rh, In, and Bi as internal standards. Samples were then analyzed for mineral content using an Agilent 7500ce Inductively Coupled Plasma- Mass Spectrometer (ICP-MS). Three modes were used to minimize spectral interferences for mineral analysis. Sulfur and Cu were analyzed using He-mode and Mo was analyzed in non-gas mode. Urine and water samples were also analyzed for S, Cu, and Mo using an Agilent 7500ce Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). Concentrations of feed, feces, water and urine were determined using an Olympus AU 640e (Olympis America Inc., Center Valley, PA). The Olympus ISE module uses ether membrane electrodes for cations sodium ($\mathbf{Na^+}$) and potassium ($\mathbf{K^+}$) and a molecular oriented PVC membrane electrode for chloride ($\mathbf{Cl^-}$). Specific cations (Na^+, K^+) and anions (Cl^-) develop an electrical potential with ions of interest according to the Nernst Equation. The electrical potential is then compared to the Internal Reference Solution (Block Scientific Inc.) and translated into voltage and then into the ion concentration of the sample.

Water usage by the 12 chambers was measured daily. Daily water consumption per steer was calculated by multiplying daily total usage by the ratio of individual chamber urine output to total urine output for the 12 chambers.

Statistical analysis

Statistical analysis was performed using PROC MIXED sub-routine of SAS 9.2 (SAS Inst., Inc., Cary, NC). Air flow (cfm) and H_2S gas data (ppm) were passed through filters using SAS 9.2 to eliminate any possible erroneous data that may have been caused from equipment malfunction or from accidental entry of calibration data. Data mean output from unfiltered compared to filtered data is provided in the appendices in Table A.1. Air flow that equaled zero

(n = 1430) and H_2S input data greater than 0.10 ppm (n = 6) was filtered out of the data set before analysis. The independent variables or class statements considered for analysis was phase, dietary treatment (**TRT**), date, and chamber. Each model statement included TRT, phase, and the phase by TRT interaction. Date and date by TRT were analyzed separately to determine day effects for H_2S and SO_2 to help determine the appropriate days to represent mean emission within a steady state. Chamber within treatment was used as the random effect for both phase and day analysis. Both S-containing gases were analyzed as output mass units (mg/d), output per S intake, and as output per DMI. Mean emissions (H_2S and SO_2), DMI, SI, and pH were all tested for normal distribution using Shapiro-Wilk's test and for homogeneous variance using a Levene's and Brown Forsythe's test. In trial 1, mean emissions for all units of H_2S and SO_2 were natural log transformed to reduce the likelihood of homoscedasticity and normality. Contrast statements were used to determine treatment differences within each phase. Statistical analysis on performance data was determined using TRT as the independent variable and average daily gain (**ADG**), DMI, body weight (**BW**), and gain to feed ratio (**G:F**) as the dependent variables. Sulfur balance was analyzed similar to performance data only changing independent variables to S in water, feed, urine, feces, gas, S retained, and percent S retained. Statistical significance was declared at a *P*-value at or below 0.05 and trends at *P*-value at or below 0.10.

RESULTS

Trial 1

In trial 1, cattle tended to gain less weight per d with the 60% DDGS diet (P = 0.06; Table 3) compared to cattle on the 40% DDGS diet. Daily weight gain on the 40% and 60% DDGS treatments were similar to control treatments. Dry matter intakes and feed to G:F were similar among treatments. Cattle on 60% DDGS diet had numerically lower DMI and G:F than the other treatments. Cattle fed the 40% DDGS diet tended to have lower digestibility compared to cattle the fed 60% DDGS diet ($P < 0.10$). Performance data provided in Table 3 was determined from the days post arrival up to the day cattle departed the air quality facility. Cattle fed all treatments were in positive energy balance and had a rate of gain typical of cattle in metabolism units (Depenbusch et al., 2009; Gunn et al., 2009). Due to natural log transformations, upper confidence limits (*UCL*) were back transformed by taking the exponent of the untranformed means plus the SEM and lower confidence limts (*LCL*) by taking the exponent of the untranformed means minus the SEM (Table 4).

Table 3.3 Effects of dry distiller's grain with soluble on performance in trial 1

	Diet				
Item	Control	40% DDGS[1]	60% DDGS[1]	SEM	*P*- value
Initial BW, kg	252	245	246	22	0.97
Final BW, kg	288	287	274	20	0.84
DMI, kg/d	6.07	6.36	5.74	0.84	0.84
ADG, kg	0.91[ab]	1.06[a]	0.71[b]	0.10	0.06
Gain: feed	0.153	0.183	0.125	0.03	0.28
DM digestibility, %	69.26[ab]	71.35[a]	63.97[b]	2.46	0.10
OM digestibility, %	70.15[ab]	71.30[a]	63.89[b]	2.48	0.09

[1] DDGS- dry distiller's grain with soluble

[a, b] Means without a common superscript within a row tend to differ ($P \leq 0.10$)

Table 3.4 Effects of distiller's grain with soluble levels on sulfur-containing emissions for phase 1 and 2 during trial 1

	Phase 1			Phase 2			*P*- value		
Item	0	40	60	0	40	60	TRT	Phase	TRT × Phase
H_2S, mg/d‡	12.28[a]	124.45[b]	60.88[ab]	-5.25	9.91	3.92	0.20	< 0.01	0.28
*UCL**	*34.92*	*171.65*	*92.24*	*12.26*	*28.58*	*21.10*			
*LCL**	*-5.23*	*86.65*	*35.77*	*-18.79*	*-5.03*	*-9.83*			
H_2S, mg/g SI	0.99	3.38	1.73	-0.46	0.2	0.20	0.24	< 0.01	0.72
UCL	*1.89*	*4.44*	*2.59*	*0.24*	*1.23*	*0.87*			
LCL	*0.20*	*2.44*	*0.96*	*-1.08*	*-0.13*	*-0.41*			
H_2S, mg/kg DMI†‡	2.15[a]	19.02[b]	9.76[ab]	-0.95	2.43	0.97	0.16	< 0.01	0.29
UCL	*5.49*	*25.83*	*14.40*	*1.54*	*5.35*	*3.55*			
LCL	*-0.47*	*13.51*	*6.01*	*-2.90*	*0.07*	*-1.11*			
SO_2, mg/d	3.75	-4.83	11.77	-19.4	-6.66	-15.5	0.95	0.08	0.46
UCL	*25.56*	*10.94*	*32.76*	*-6.19*	*10.60*	*-3.10*			
LCL	*-12.15*	*-16.83*	*-4.20*	*-29.04*	*-19.37*	*-24.96*			
SO_2, mg/g SI	0.26	-0.11	0.29	-1.01	-0.09	-0.10	0.81	0.29	0.57
UCL	*1.24*	*0.63*	*1.14*	*-0.26*	*0.66*	*0.64*			
LCL	*-0.50*	*-0.70*	*-0.38*	*-1.56*	*-0.69*	*-0.69*			
SO_2, mg/kg DMI	0.56	-0.80	1.76	-3.68	1.72	-2.74	0.79	0.25	0.30
UCL	*4.86*	*2.28*	*5.86*	*-1.39*	*6.49*	*-0.44*			
LCL	*-2.35*	*-3.00*	*-1.17*	*-5.23*	*-1.54*	*-4.39*			

[a, b] Means without common superscripts within a phase differ ($P \leq 0.05$)

† Means tend to show a linear effect within a phase ($P \leq 0.10$)

‡ Means tend to show a quadratic effect within a phase ($P \leq 0.10$)

* Upper and lower confidence limits (*UCL, LCL*) express reliability of the estimated mean emissions. The mean emissions were all back-transformed from a natural log transformation to satisfy normal distribution and equal variance

Phase 1

Mean emissions reported for phase 1 were determined using data from d 11 to d 14 which represent steady state emissions (Table 3.4). Day effects were highly significant on S-containing emissions during phase 1 (Table A.2). Emissions across treatments had similar daily variability with no significant day by treatment interaction observed. Days selected to report means is further illustrated in the appendices in Figures A.1-A.6.

The phase by treatment interactions were not significant for all parameters measured. Consequently, the main effects of treatment and phase will be discussed separately. Treatment differences are shown graphically in Figures A.7-A.12. Hydrogen sulfide emissions expressed in mass units and per unit of DMI were greater for DGS diets as compared to the control (Table 3.4). There was a tendency ($P < 0.10$) for a quadratic response with the 40% DDGS diet having the highest H_2S emissions. Hydrogen sulfide expressed in mass units per unit of DMI also expressed tendency ($P < 0.10$) for a linear response. Sulfur dioxide was similar among treatments.

Phase 2

Mean emissions were reported (Table 3.4) from data collected on the last 2 d of the trial (d 16 and 17) to best report emissions within a steady state (Figures A.1-A.9). A day effect was reported for H_2S emissions during phase 2 but treatment by day interaction was not significant (Table A.2).

Separation of urine and feces significantly decreased H_2S emissions ($P < 0.01$) when expressed in mass units per d or as a function of SI and DMI. This suggests that most of the H_2S generated in phase 1 originated from the manure mixture and very little from enteric

fermentation in the animal. Feed, water, feces, and urine collections along with gas data during the last 2 d of trial 1 were used to estimate total sulfur balance by comparing sulfur intake (**SI**) in feed and water to sulfur expelled in the form of feces and urine. Sulfur intake increased (P = 0.01) with greater inclusion levels of DDGS (Table 3.5). Water intake was calculated as a function of urine output to predict the contribution of water SI to total SI. Sulfur levels in feed were greater in the 40% and 60% DDGS diets compared to control diets ($P < 0.01$). Expelled S in urine and feces showed a similar trend as S intake increased as levels of DDGS increased. Cattle fed 40% and 60% DDGS diets had greater sulfur in urine ($P < 0.01$) as compared to the control diets. The amount of S retained on a mass unit basis as well as expressed as a percentage was similar among treatments. A study conducted from Bouchard and Conrad (1972) found that feeding dietary S above 0.3% of the diet DM using various types of sulfates resulted in greater S retention. Feeding S at levels of 0.24% using K_2SO_4 + $MgSO_4$ and Na_2SO_4 as sources of supplemental sulfates resulted in retention levels in tissure near 40% and 30% in dairy cattle, respectively (Bouchard and Conrad, 1972). Our results offer similar retention levels in cattle fed high S diets; however, we did not see differences in the percent of S retained between low and high S diets. Increasing levels of DDGS in cattle diets showed a numeric reduction in the percent of S retained. The control diets had the lowest apparent digestibilty (53.47%) compared to the 40% and 60% DDGS diets, 74.94% and 72.75% respectively (P = 0.02). Our results for the percent of apparent S digested agree with other studies. Boila and Golfman (1991) reported apparent digestibility of S to be around 75% for diets with both low and high levels of S, 1.3 and 3.9 g/kg DM respectively. Previous work from Bouchard and Conrad (1973) reported apparent S digestibility in the range of 70% to 80% for cattle fed 0.18% to 0.24% dietary S. Within the

current study, cattle on the control diet were fed 0.22% dietary S but only had a S digestibility of 53.47%.

The acidity of manure (feces and urine mixture) in phase 1 increased ($P = 0.03$) as the level of dietary DDGS increased (Table 3.6). In phase 2, pH of fecese tended to be greater for the 40% DDGS treatment than for the control or 60% DDGS diet ($P = 0.08$). Urine pH was similar among treatments. Aerobic and anaerobic digestion of organic residues in manure by bacteria may result in sulfate reduction, contributing to emissions H_2S and along with other S compounds (Mackie et al., 1998; Spiehs and Varel, 2009). Inside the rumen, sulfides are readily protonated within a pH range of 5.5 to 7.2 (Kung, 2008). It would be reasonable to presume that forms of SRB in manure would favor environments within or near the same pH range. Manure collected during phase 1 was the main source that contributed to H_2S emissions. However, manure pH was near or above pH 8. This pH increase compared to feces collected during phase 2 may be a result of sulfate reduction and S protonation.

Table 3.5 Effects of distiller's grain with soluble on total sulfur balance for trial 1 during phase 2

	Diet					Contrast	
Item	Control	40% DDGS	60% DDGS	SEM	*P*- value	Linear	Quadratic
Feed S intake, g/d	13.25[a]	34.90[ab]	44.47[b]	6.08	0.01	< 0.01	0.90
Water S intake[1], g/d	0.07[a]	0.16[ab]	0.19[b]	0.02	0.02	< 0.01	0.53
Total S intake, g/d	13.34[a]	35.06[ab]	44.64[b]	6.08	0.01	< 0.01	0.90
Urinary S, g/d	1.73[a]	12.52[b]	19.54[b]	2.69	< 0.01	< 0.01	0.72
Fecal S, g/d	6.20[a]	7.12[a]	11.52[b]	1.15	0.01	0.01	0.07
S emissions[2], g/d	-0.0125	0.0053	0.0031	0.017	0.72	0.47	0.70
Total S excreted, g/d	7.92[a]	19.65[b]	31.07[c]	2.56	< 0.01	< 0.01	0.22
S digestibility[3], %	53.47[a]	75.94[b]	72.75[ab]	5.21	0.02	0.01	0.15
S retained, g/d	5.43	15.41	13.58	4.25	0.24	0.14	0.35
S retained, %	40.64	37.35	29.55	9.26	0.65	0.43	0.70

[1] Water S intake was calculated as a function of urine output

[2] Sulfur emissions during phase 2 were not different from zero

[3] Digestibility calculated by 1- (fecal S/total S intake) × 100

[a, b, c] Means without common superscripts within a row differ

* Sulfur balance was determined from data collected during phase 2 (2 d)

Table 3.6 Effects of feeding distiller's grain with soluble on excreta pH and output during trial 1

	Diet					Contrast	
Item	0% DDGS	40% DDGS	60% DDGS	SEM	*P*- value	Linear	Quadratic
Manure pH[1]	7.73[a]	8.46[ab]	8.76[b]	0.24	0.03	0.01	0.89
Feces pH[2]	6.00[a]	6.64[b]	6.15[ab]	0.20	0.08	0.34	0.04
Urine pH[2]	8.67	7.42	6.84	0.62	0.14	0.05	0.97
Dry fecal output[2], kg/d	1.98	1.80	2.11	0.23	0.57	0.82	0.32
Urine output[2], L/d	2.42	5.26	6.69	1.40	0.13	0.05	0.99

[1] Manure- collected during phase 1; mixture of feces and urine

[2] Feces and urine- collected separately during phase 2

[a, b] Treatment means within a row without a common superscript differ

Trial 2

The purpose of trial 2 was to introduce appropriate levels of Mo and Cu into the diet to bind dietary S before reduction to hydrogen sulfide occurred from ruminal SRB. Molybdenum was added in the form of Na_2MoO_4 and Cu was provided through supplemental $CuCl_2$ in the 40% DDGS+ diets. Sodium molybdate and $CuCl_2$ were included to provide an addition 6 ppm Mo and 60 ppm Cu, respectively. However, TMR analyses would indicate that 8 ppm Mo and 90 ppm Cu were included in the 40% DDGS+ diet. The effects of dietary treatments on animal performance are shown in Table 3.7. Cattle were in positive energy balance and consuming more than 2% of their BW. Growth and feed conversion efficiency were similar among treatments. Molybdenum and Cu levels were greater ($P < 0.01$) for the DDGS+ diet. Dry matter digestibility ($P = 0.07$) tended to be greater for cattle fed the control diet compared to the cattle fed 40% DDGS+ diet.

Sulfur emissions were measured as mass units (mg/d), mass units per unit of SI (mg/g SI), and as mass units per unit of DMI (mg/kg DMI). For all measures of H_2S and SO_2, separation of feces and urine in phase 2 significantly decreased emissions ($P < 0.01$; Table 3.8). As shown in trial 1, very little of the H_2S emitted came directly from the animal but originates from the manure in the fecal pans.

Table 3.7 Effects of dry distiller's grain with soluble fortified with copper and molybdenum on performance in trial 2

	Diet				
Item	Control	40% DDGS[1]	40% DDGS+[2]	SEM	*P*- value
Initial BW, kg	300	313	312	16	0.82
Final BW, kg	342	351	339	16	0.86
DMI, kg/d	8.07	7.12	7.49	0.47	0.40
ADG, kg	1.08	0.96	0.70	0.16	0.30
Gain: feed	0.132	0.131	0.091	0.02	0.20
DM digestibility, %	73.90[a]	68.34[ab]	65.49[b]	2.23	0.07
OM digestibility, %	74.41[a]	69.57[ab]	66.66[b]	2.35	0.11
Mo, ppm	1.63[a]	1.38[a]	9.49[b]	0.40	< 0.01
Cu, ppm	12.89[a]	22.29[a]	111.51[b]	4.84	< 0.01

[1]DDGS- dry distiller's grain with soluble

[2] 40 % DDGS+ is supplemented with 8 ppm Mo and 90 ppm Cu

[a, b] Means without a common superscript within a row tended to differ

Table 3.8 Effects of dry distiller's grain with soluble fortified with copper and molybdenum sulfur-containing emissions for phase 1 and 2 during trial 2

	Phase 1			Phase 2				*P*- value		
Item	0	40	40 +	0	40	40 +	SEM	TRT	Phase	TRT × Phase
H_2S, mg/d	58.25[a]	514.84[b]	419.36[b]	-30.25	-24.56	-2.65	-	< 0.01	< 0.01	< 0.01
*UCL**	*88.50*	*570.02*	*470.20*	*-10.32*	*-3.93*	*20.47*	-			
*LCL**	*29.36*	*462.02*	*370.87*	*-47.83*	*-42.84*	*-23.42*	-			
H_2S, mg/g SI	3.56[a]	11.78[b]	8.53[b]	-2.54	-0.58	-0.05	1.14	< 0.01	< 0.01	0.03
H_2S, mg/kg DMI	6.76[a]	65.48[b]	51.07[b]	-5.22	-3.26	-0.72	-	< 0.01	< 0.01	< 0.01
UCL	*10.24*	*72.22*	*57.18*	*-2.94*	*-0.74*	*2.07*	-			
LCL	*3.55*	*58.74*	*44.96*	*-7.22*	*-5.50*	*-3.24*	-			
SO_2, mg/d	3.80	15.71	20.55	-14.64	-7.42	-18.88	6.95	0.46	< 0.01	0.11
SO_2, mg/g SI	0.19	0.34	0.39	-1.06[b]	-0.23[c]	-0.44[c]	0.20	0.05	< 0.01	0.32
SO_2, mg/kg DMI	0.40	1.98	2.40	-2.13	-1.17	-2.49	0.84	0.43	< 0.01	0.06

[a, b] Means without common superscripts within a phase differ ($P \leq 0.05$)

* Upper and lower confidence limits (UCL, LCL); exponent of untransformed mean ± 1 SEM, express reliability of the estimated mean emissions. The mean emissions were all back-transformed from a square root transformation to satisfy normal distribution and equal variance. Standard error of the mean (SEM) is expressed for all untransformed means

Phase 1

Emissions during trial 2 for phase 1 were determined using a 4 d measurement interval (d 20 to d 23) which represented a pseudo-steady state condition (Table 3.8). Day variability was highly significant in all measures of emissions for both H_2S and SO_2 (data not shown), however the treatment by day interaction was non-significant for all expressed measurements. Daily emission plots for both H_2S and SO_2 are provided in the appendices (Figures A.13-A.18) with days used for each phase reported. Bar graphs are provided in the appendices for visual comparisons of treatment means within each phase (Figures A.19-A.24).

A treatment by phase interaction was evident for H_2S expressed in mass units ($P < 0.01$), mass unit per SI ($P = 0.03$), and mass unit per DMI ($P < 0.01$; Table 3.8). Addition of DDGS increased H_2S emissions for all units measured ($P < 0.01$) compared to emissions from cattle fed the control diet. Comparing an orthogonal contrast between 40% DDGS and 40% DDGS+ treatment would indicate a trend ($P = 0.08$) for a 27% reduction in H_2S emissions with the addition supplemental Cu and Mo. A 22% reduction in H_2S emissions was also seen when mass unts were adjusted for DMI with the 40% DDGS+ treatment; however, this difference was not significant.

Phase 2

Emissions reported during trial 2 for phase 2 were determined from data collected on d 26 to d 29 (Table 3.8). Both H_2S and SO_2 produced negative emissions during phase 2. This is likely caused by a greater in-air gas concentration compared to gas concentrations ouput from the

chambers. Lower concentrations within the chamber rooms compared to in-air concentrations may be a result of emissions being near or below the detection limit of the analyzer. It is possible that some H_2S was being removed from the air via inhalation and absorption within the animal. Sulfur dioxide emissions were less ($P < 0.05$) for the 40% DDGS diets compared to the control diets on a mass unit per SI basis. However, caution should be observed when interpreting these results as treatment means were all negative.

Feed, water, feces, and urine collections along with gas data during the last 4 d of trial 2 were used to estimate total S balance by comparing SI in feed and water to S excreted in the form of feces, urine, and gas. Sulfur loss as gas was not different from zero during phase 2. Dietary sulfur intake was greater for the 40% DDGS diets compared to S intake from steers on the control diet ($P < 0.01$; Table 3.9). Similarly, excreted sulfur in urine ($P < 0.01$) and feces ($P = 0.03$) were also greater for both 40% DDGS diets compared to control diets. Sulfur in gas form was emitted at low levels and contributed little to total expelled S. Amount of retained S was determined by all unaccounted sulfur. Both DDGS diets had greater retained S (g/d) compared to control diets ($P < 0.01$). Addition of Cu and Mo to the 40% DDGS diet did not lower S excreted in feces or urine. It did slightly increase S emissions ($P = 0.03$) but the magnitude was very small (not different from zero) relative to the amount S consumed and excreted in feces and urine. Sulfur retention was similar among 40% DDGS treatments but differed the control diets on mass unit basis ($P < 0.01$) and a percent of S retained ($P = 0.03$). Sulfur digestibility was greater ($P < 0.01$) for both DDGS diets as compared to the control diets as well. One can infer from this observation the S in DDGS is more available than S in the basal or control diet.

Fecal (P = 0.01) and manure (P = 0.02) pH were greater for both 40% DDGS diets compared to control (Table 3.10). Similar to the results from trial 1, the manure mixture had a greater pH compared to the pH of feces or urine.

Table 3.9 Effect of distiller's grain with soluble fortified with copper and molybdenum on total sulfur balance for trial 2

	Diet				
Item	Control	40% DDGS	40% DDGS+	SEM	*P*- value
Feed S intake, g/d	11.51[a]	35.41[b]	38.91[b]	2.11	< 0.01
Water S intake, g/d	0.18	0.19	0.22	0.03	0.55
Total S intake, g/d	11.69[a]	35.59[b]	39.14[b]	2.12	< 0.01
Urinary S, g/d	3.35[a]	19.85[b]	19.80[b]	1.70	< 0.01
Fecal S, g/d	7.40[a]	9.12[b]	9.37[b]	0.47	0.03
S emissions[1], g/d	-0.036[a]	-0.027[ab]	-0.011[b]	0.005	0.03
Total S expelled, g/d	10.71[a]	28.94[b]	29.16[b]	1.86	< 0.01
S digestibility[2], %	34.94[a]	74.25[b]	75.94[b]	3.28	< 0.01
S retained, g/d	0.95[a]	6.63[b]	9.96[b]	1.38	< 0.01
S retained, %	8.1[a]	18.6[ab]	25.5[b]	4.0	0.03

[1] Sulfur emissions were not different from zero, despite apparent treatment differences

[2] Digestibility calculated by 1- (fecal S/total S intake) × 100

[a, b] Means without common superscripts within a row differ

* Sulfur balance was determined from data collected during phase 2 (4 d)

Table 3.10 Effects of feeding distiller's grain with soluble on excreta pH and output during trial 2

	Diet				
Item	0% DDGS	40% DDGS	40% DDGS+	SEM	*P*- value
Manure pH[1]	7.23[a]	8.21[b]	8.09[b]	0.20	0.01
Feces pH[2]	4.92[a]	5.70[b]	5.56[b]	0.16	0.02
Urine pH[2]	8.05	6.64	6.63	0.52	0.14
Dry fecal output[2], kg/d	2.22	2.29	2.63	0.20	0.35
Urine output[2], L/d	3.81	3.99	4.14	0.73	0.95

[1] Manure- collected during phase 1; mixture of feces and urine

[2] Feces and urine- collected separately during phase 2

[a, b] Treatment means within a row without a common superscript differ

Table 3.11 Hydrogen sulfide gas emitted per gram of sulfur intake during phase 1 of each trial

	Trial 1			Trial 2		
Item	Control	40% DDGS	60% DDGS	Control	40% DDGS	40% DDGS+
H_2S, mg/d	12.28	124.45	60.88	58.25	514.84	419.36
S Intake, mg/d	12711	38971	46165	17163	43278	50215
H_2S per S intake, %	0.10	0.32	0.13	0.34	1.19	0.84

DISCUSSION

In trial 1, the objective was to determine if S-containing gases such as H_2S and SO_2 would increase from cattle due to greater inclusion levels of DDGS within the diet. Feeding increased levels of DDGS would provide greater levels of protein or other S-containing organic matter in cattle diets compared to the cattle fed the control diet. Greater dietary S could elevate levels of emitted H_2S in the manure, which creates a greater risk during manure handling and removal to both humans and animals (Hooser et al., 2000). Additionally, work from Bull and Vandersall (1973) and Spears et al. (1977) suggests that ruminal digestion may be affected by S levels within the diet. Within phase 1 of trial 1, dietary SI increased from 13 g/d for the control diet, to 39 g/d for the 40% DDGS diet, and 46 g/d for the 60% diet, respectively (Table 3.11). However, increasing levels of dieatary S did not increase S-containing emissions when comparing 40% DDGS diets to 60% DDGS diets. This may suggest a limited capacity for S reduction in the rumen which was exceeded with the 60% DDGS diet. Cattle fed 40% DDGS diets had the greatest percent of SI converted to H_2S gas. Feeding DDGS diets above 40% may not increase S-containing gas production due to affects on ruminal fermentation and reduction in DMI. Comparison of daily H_2S emissions from trials 1 and 2 (Table 3.11) show a much greater H_2S emission per unit of SI, which suggests that ruminal adaptation may be occurring. It is possible the SRB are increased in number, efficiency, or both as the length of exposure to high S diets increases. It is also reasonable to speculate the length of time with exposure to low pH generated in high concentrate fed animals may facilitate sulfate reduction. This may also explain the declined performance measures from cattle fed 60% DDGS.

Majority of S-containing emissions were contributed from H_2S gas, while sulfur dioxide emissions were not different from zero for both phase 1 and 2. The significant decrease of H_2S emissions when urine and feces were collected separately inside the chamber (phase 2) would suggest that very little, if any, S-containing gases formed inside the rumen escape by eructation; and that mixture of urine and feces is the main source of S-containing emissions, particularly H_2S gas. Daily variance of H_2S emissions may have been an effect of agitation of manure inside the collection vessel from cattle defecating or stepping back into the collection vessel. Hydrogen sulfide has a low solubility in water and therefore will mostly remain trapped in bubbles in the manure (Pickrell, 1991; Hooser et al., 2000); however, H_2S escapes rapidly when manure is agitated (Osweiler et al., 1985; Donham et al., 1988). When looking at emissions within each day, H_2S spikes were consistantly recorded around 6:00am (data not shown), the time at which manure was removed to maintain a 2 in residual base in the pan.

The use of 8 ppm Mo and 90 ppm Cu as a supplemental feed additive to decrease S-containing gases emitted from the animal suggested this strategy may be effective for reduction of H_2S emissions, particularly decreasing the proportion of SI that is converted to H_2S gas. When H_2S emissions were adjusted on SI, a 27% decraese was detected between emissions from cattle on 40% DDGS diets compared to cattle on 40% DDGS+ diets supplemented with an additional Mo and Cu ($P = 0.08$). These results provide evidence that a reaction with dietary S may be forming tetrathiomolybdate (MoS_4^{2-}), which readily reacts with Cu to form an insoluble compound, MoS_4Cu (Hamsell et al., 2010).

In other studies that measured H_2S in the ruminal gas cap, increased concentrations were detected in cattle fed diets high S diets (Gould et al., 1997; Loneragan et al., 1998). Loneragan et al. (1998) reported H_2S concentrations in the ruminal gas cap as high as 18.77 mg/L or 13,500 ppm in cattle fed high S diets. Inhalation of eructated gas into the lungs is the likely route of H_2S absorption from cattle as protonated sulfides are unable to be absorbed through the rumen wall (NRC, 2005; Crawford, 2007). Other reports have approximated 60% of eructated gases are inhaled and enter the respiratory tract (Bulgin et al., 1996). In phase 2 of both trials, H_2S gas was decreased to undetectable levels. These results would suggest that almost all H_2S emissions recorded in phase 1 resulted from the mixture of urine and feces in the collection pan and not by eructation from the cattle.

Maintaining a resident population of SRB in the fecal pan contributed to the elevated emissions of H_2S gas. The collection system used during phase 1 of each trial may more accurately portray emissions from liquid manure handling and storage systems. Manure (feces and urine) mixture during phase 1 was the main source of H_2S emissions recorded within the chamber rooms. Molecular H_2S begins to dissociate to HS^- or S^{2-} in liquid at pH 8 or higher. As more sulfide is protonated and released as H_2S gas, manure pH increases (Thistlethwayte, 1972; Xue et al., 1998). Results from both trials agree with these reports that elevated manure pH increases as manure S is protonated and emitted in the form of H_2S. Additionally, temperature also influences protonation of sulfides similar to pH; a temperature increase of 1° C has the same effect as increasing the pH by 0.15 (Thistlethwayte, 1972; Xue et al., 1998). In the current study,

the averge room temperature had a set point around 16° C and did not deviate much from the set point temperature. Therefore, temperature had likely little effect to the higher H_2S emissions reported and greater percent of dietary S emitted as H_2S in trial 2 compared to trial 1 (Table 11).

Sulfur balance for trial 1 and trial 2 provided different retention levels in mass units and as a percent of total S intake. Bioavailability of dietary S is reported to be dependent on total S intake and form of S (Fron et al., 1990). Furthermore, available nitrogen (**N**) in the diet may be metabolized resulting in greater levels of S excreted as sulfate and total S concentration in urine (Salsbury et al., 1971; Fron et al., 1990). Increased levels of DDGS would have increased levels of N in the diet, which could influence S retention. Bouchard and Conrad (1972) reported the greatest S retention levels in cattle that were fed a lower N:S ratio. This fits with the retention differences reported in trial 2. The calculated N:S was 11.1 for the control diet compared to 6.1 for the 40% DDGS diet and 6.6 for the 40% DDGS+ diet (data not shown).

Further studies would be beneficial to confirm the impact Mo and Cu have on cattle diets with high levels of DDGS, and if they do in fact reduce H_2S emissions. Results from our study suggest that manure contributes much of the S-containing emissions. More research on the source of emissions could serve as useful information for future research to help determine whether it would be better to treat the animal diet or if possible application into manure storage systems would be a better strategy for overall reduction of S-containing emissions.

CHAPTER 4: EFFECTS OF DISTILLER'S GRAIN WITH SOLUBLE AND SUPPLEMENTAL COPPER AND MOLYBDENUM ON NITROGENOUS EMISSIONS AND NITROGEN RETENTION

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SUMMARY

When moderate to high levels of DGS are fed, dietary CP is elevated, which may contribute to environmental pollution from increased nitrogenous emissions. A study was conducted to evaluate the affects of DDGS on NH_3 and other N-emissions. Two trials were conducted within this study using 12 Holstein steers housed in individual environmentally-controlled rooms to monitor gas production. Three dietary treatments were fed in trial 1; containing 0% (control), 40%, and 60% DDGS. In trial 2, treatments were the same except the 60% DDGS dietary treatment was replaced with a 40% DDGS diet fortified with 8 ppm Mo and 90 ppm Cu, which will be referred to as 40% DDGS+. Each trial was divided into 2 phases; phase 1 of each trial monitored emissions when urine and feces were collected in the same vessel (manure mixture). Phase 2 of trial 1 monitored emissions for 2 d while phase 2 of trial 2 monitored emissions for 4 d while steers were fitted with fecal bags to separate feces from urine. In trial 1, N_2O showed a linear reduction as inclusion of DDGS increased from 0, 40, to 60% of the DM diet ($P = 0.02$) during phase 1. In trial 2, NH_3 emissions tended to increase with inclusion of 40% DDGS compared to the control diet when expressed as mass units ($P = 0.03$), mass units per DMI ($P = 0.06$), and mass units per N intake ($P = 0.10$). Separation of feces and urine during phase 2 of both trials significantly decreased NH_3 emissions ($P < 0.01$) while increasing NOx emissions ($P < 0.01$). Nitrogen balance for both trial 1 and trial 2 shows that

DDGS tends to improve N digestibility compared to the control diet *(P* < 0.05). Molybdenum and Cu treatment did not have an effect on N-emissions, N digestibility, or N retention.

Key words: distiller's grain with soluble, ammonia, nitrogen, molybdenum, copper

INTRODUCTION

Distiller's grain with soluble in feedlot diets increases crude protein (**CP**) levels along with other nutrients relative to conventional feedlot diets. Distiller's grain with soluble is nearly 3-fold more concentrated in protein, fat, fiber, and other minerals (Stock et al., 2000). This increase in nutrient concentration in DGS is the result of removing the starch from the corn kernel during the dry milling process for ethanol production (Klopfenstein et al., 2008). The greater concentration of protein in diets containing high levels of DGS may contribute to an increase in ammonia (**NH_3**) and other aromatic compounds that are formed by aerobic and anaerobic digestion of organic residues by bacteria (Mackie et al., 1998; Spiehs and Varel, 2009). Typically, increasing dietary **N** will increase urinary N excretion (Gueye et al., 2003; McBride et al., 2003). The amount of urinary N excretion (Erickson et al., 2001) and the urine pH are determinants of the amount of N-emissions (Luebes et al., 1974; Cole et al., 2005).

In addition to increasing dietary N, DGS also increases levels of **S** in cattle diets. A study from Rumsey (1978) suggests that increasing S content may reduce ruminal pH and ruminal concentration of NH_3. Introducing supplemental **Mo** and **Cu** to high S diets may limit the availability of S in the rumen. Dietary S and S in protein react with Mo to form tetrathiomolybdates (**MoS_4^{2-}**) that then react with Cu and particulate matter in the rumen, forming a highly stable compound that is very difficult for the animal to digest and absorb (Gould et al., 2002).

In trial 1, cattle were fed dry distiller's grain with soluble (**DDGS**) at 0, 40, and 60% to determine the effects on NH_3 and other N-emissions. The purpose of trial 1 was to determine if increased concentrations of dietary N through increased inclusion levels of DDGS would cause

increased emissions of NH_3 and other nitrogenous gases such as nitrous oxide (**N_2O**), and nitrogen oxides (**NOx**; includes NO_2 and NO^-).

In trial 2, dietary treatment of 60% DDGS was replaced with another 40% DDGS diet containing 8 ppm molybdenum and 90 ppm copper. Molybdenum and Cu bind only to degraded S from protein and inorganic S from the diet or saliva. Therefore, degradable N may be influenced by the availability of S in the rumen (Suttle, 1991). Including 8 ppm Mo and 90 ppm Cu with 40% DDGS in cattle diets may influence NH_3 and other N-emissions.

MATERIALS AND METHODS

Gas collection

Two studies were conducted at Michigan State University at the Animal Air Quality Research Facility (**AAQRF**) to address concerns associated with high inclusion levels of DDGS. Approval for this study was provided by the Michigan State University Animal Care and Use Committee (AUF # 07/09-110-00). Individual Environmentally-controlled rooms at AAQRF monitor incoming and outgoing air from 12 rooms for concentrations of NH_3, N_2O, and NOx gases. Steers were housed in chamber rooms to determine whether increased DDGS in the diet would increase N- emissions. Before arrival to AAQRF, steers were held at Michigan State University's Beef Cattle Teaching and Research Center (**BCTRC**) where they were weighed and vaccinated for prevention of clostridial disease using Ultrabac-7 (Pfizer, New York, NY) and respiratory infections (Bovi-Shield GOLD® 5 Pfizer, New York, NY). At trial initiation; a 2 wk adjustment time was allotted before steers were transported to AAQRF. During this time, steers (n = 12) were started on a corn-based concentrate diet (control diet). After the first week, steers (n = 8) were randomly assigned to a 40% DDGS diet. The control diet consisted of 81% high moisture corn (**HMC**), 10% corn silage (**CS**), 5% soybean meal (**SBM**), and 4% mineral supplement. The 40% DDGS diet consisted of 40% DDGS, 46% HMC, 10% CS, and 4% mineral supplement. Steers were broke to lead to insure safe handling and limit stress on the animal during the study at AAQRF. Four days prior to arrival at AAQRF, steers were housed in metabolism stalls at BCTRC to adapt them to similar living conditions that they would have in the individual, environmentally-controlled rooms at AAQRF. Steer weights were taken before feeding on 2 consecutive days before transportation to AAQRF. Weights were also taken on 2

consecutive days upon return to the feedlot facility. Steers (n=12) were randomly assigned to 3 dietary treatments prior to arrival at AAQRF.

Two different trials were conducted at AAQRF to determine the effects of increasing DDGS on animal performance and N emissions. In both trials, 12 Holstein steers were placed into environmentally-controlled rooms with 4 steers per dietary treatment. The dietary treatments for trials 1 and 2 are shown in Table 4.1. In trial 1, 3 levels of DDGS were fed (0, 40, and 60%) and 2 levels in trial 2 (0 and 40%). In trial 2, the third treatment was 40% DDGS fortified with 8 ppm Mo and 90 ppm Cu. The latter treatment was called 40% DDGS+ (Table 2). The source of Mo was sodium molybdate ($\mathbf{Na_2MoO_4}$) and the Cu source was copper chloride ($\mathbf{CuCl_2}$). Copper chloride was selected to minimize the amount of additional S in the diet. All diets were top dressed daily with thiamine (200 mg) as a preventive step against S toxicity. A 1 min eye and ear twitch count was recorded daily to monitor physical symptoms associated with PEM. Recorded twitches for each steer were unchanged during both studies. On d 5 of trial 1, a steer on the control diet had to be pulled off the study due to poor feed intake and formation of hematoma near the left hook bone.

Table 4.1 Dietary ingredients and composition for trial 1

	Trial 1			Trial 2		
Ingredient, % of DM	Control	40	60	Control	40	40 +[2]
DDGS[1]	-	40	60	-	40	60
High moisture corn	81	46	26	81	46	26
Corn silage	10	10	10	10	10	10
Soybean meal	5	-	-	5	-	-
DGS supplement	-	-	-	-	4	4
BFS50 supplement	4	4	4	4	-	-
Total	100	100	100	100	100	100
			Diet composition			
Dry matter, %	67.7	74.8	76.0	68.6	73.6	74.5
			% of DM			
Ash	5.6	7.4	8.0	4.4	6.4	7.1
ADF	5.9	7.1	8.8	4.2	7.2	7.0
NDF	11.8	19.0	23.2	11.7	19.9	19.6
Starch	60.3	33.5	22.1	58.6	33.5	33.3
Ether extract	2.9	4.6	5.5	2.2	3.9	3.9
Crude protein	12.9	21.5	24.6	12.9	19.1	18.8
ADIP[3]	1.5	5.5	6.6	1.3	4.5	5.0
Gross energy, Mcal/Kg	4.0	4.3	4.4	4.4	4.7	4.7

Ingredient, % of DM	BFS50 supplement	DGS supplement
Akey TM premix # 4[TM]*	1.4	2.4
Limestone	24.9	71.5
Soybean meal, 48% N	48.3	-
Rumensin[TM] 80	0.3	0.4
TM salt	9.6	18.0
Vitamin E, 5%	0.2	0.1
Urea, 45% N	9.6	7.6
Potassium chloride	5.1	-
Selenium 90	0.7	-
Total	100	100

[1] DDGS- dry distiller's grain with soluble

[2] 40% + is fortified with supplemental 8 ppm molybdenum and 90 ppm copper

[3] Acid detergent insoluble proteins (ADIP) represent a portion of undegradable intake protein (UIP) that is completely indigestible to the cattle

* Akey TM premix # 4 composition: 9% Mg, 4% S, 0.02% Co, 1% Cu, 0.09% I, 2% Fe, 4% Mn, 0.03% Se, 4% Zn, 4,400,000 IU vitamin A, 550,000 IU vitamin D, and 5,500 IU vitamin E/kg (Akey Inc., Lewisburg, OH)

Steers were adapted to diets and chamber rooms for 10 d (trial 1) prior to collecting emissions data and1 d in trial 2. Emissions were recorded over 14 d in trial 1 and 22 d in trial 2. Emissions data from the last 4 d were summarized and reported as emissions during phase 1. Temperature in the chambers was maintained near 16° C and air flow was closely regulated near 17 m^3/min or 40 air changes per hour. Humidity in each chamber room was recorded but was not regulated. Sampling cycle of the 12 rooms plus the background air would take 3 h 15 min and allowed for 7 to 8 observations per room per d. During the 15 min sampling cycle, the first 9.5 min were used as a purging period and gaseous content of air was recorded the last 5.5 min. Exhausted air was sampled within an aluminum duct in each room where Teflon coated sample lines would draw air to the gas analyzers by positive static pressure from each individually sealed chamber room. Each room was also fitted with an in-house manufactured type-J thermocouple, and a Campbell Scientific ^{TM} HMP45C (Logan, Utah, Campbell Scientific, Inc.) temperature and relative humidity probe. Ammonia along with NOx emissions were measured using a TEI17C chemiluminescence analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Chemiluminescence is dependent on the reaction of NO^- and ozone (**O_3**) to form NO_2 and O_2 as a product. The reaction produces infrared light that is proportional to the concentration of NO. To determine NOx gases, NO_2 must be transformed to NO^- using a Mo-converter heated at approximately 325° C. Finally, total nitrogen (**N_t**) is measured by converting NOx and NH_3 gases to NO^- within a steel converter heated to 825° C. Once NOx and N_t concentrations are measured, NOx can then be subtracted from N_t to provide a concentration for NH_3 gas (TEI17C operator's manual). Nitrous oxide gas was monitored using an Innova Photoacoustic Field Gas-

Monitor 1412 (LumaSense Technologies, Ballerup, DK). Photoacoustics measures the amplitude of the acoustic wave. Electrical signals are generated from the microphones and amplified before being sent to an analogue-to-digital converter. The digitized signal is then converted to a gas concentration using appropriate calibration factors stored in the analyzer (Innova Airtech Instruments A/S, Ballerup, DK). Gas sampling and data collection were all computer controlled by LabView 8.2.1 software and the FieldPoint system (National Instruments, Austin, TX, USA). Analyzers output concentration was measured in ppm and was transposed into units of mg/min (Table 4.2). The units were then adjusted to 24 h and expressed as a mass output, output adjusted for N intake (**NI**), and output adjusted for dry matter intake (**DMI**).

Table 4.2 Gas unit conversion chart

A. Air Flow @ STP	1.	STP	(((temp,° C) + 273° K) × 0.967 atm)
	2.	m^3/min	STP × air flow, m^3/min
	3.	L/min	(Air flow @ STP, m^3/min × 1000 L/ m^3)
B. Emitted gas	1.	ppm	Expired, ppm – incoming, ppm = emitted, ppm
	2.	Units cancel	Emitted gas, mg/kg × 0.000001 kg/mg
C. Volume of ideal gas @ 0° C & 760 mm Hg	1.	mg/L	MW, g/mol/(22.414 L/mol) × 1000 mg/g
Adjusted equation for all gases		mg/min	(A.3) × (B.2) × (C.1)

Feed, feces and urine collection

During each gas collection period for trial 1 and 2, daily samples of total mixed ration (**TMR**) and feed weigh-back (**orts**) from each steer were collected and immediately placed in a freezer for storage. Orts were monitored daily to adjust the amount of TMR offered to 110% of the previous d composition. All feed samples and orts were later freeze-dried and ground through a Wiley Mill (Thomas Scientific) fitted with a 2 mm screen followed by a 1 mm screen. Stalls were set on an incline to allow urine to flow back and be collected in the manure pan as well as excreted feces. The feces and urine mixture was maintained at a 5 cm depth inside the pan during phase 1 of each trial. Partial removal of excreta was performed daily to maintain a 5 cm depth in the pan. The removed excreta were weighed to calculate total excretion. A 0.5 kg mixed sample of excreta was collected during each removal and immediately stored in a freezer for later analysis. Excreta removal was collected during the morning shift, whereas feeding along with TMR and ort collections were conducted in the afternoon. During each shift, chamber rooms and stalls were thoroughly cleaned and chamber rooms were only entered if they were 5 chambers or more ahead of gas sampling.

Feces and urine segregation using fecal bags

On d 15 of trial 1 and d 23 of trial 2, all manure and urine was removed and weighed from each fecal pan. Fecal bags were placed on each steer and clean pans were put back in place to collect urine only. Fecal and urine segregation took place during the last 2 d of trial 1 and the last 4 d of trial 2. This period of each trial will be referred to as phase 2. Urine pans were pulled twice daily and filtered through a screen before urine weight and volume were recorded. Two 1% aliquots were taken of the composited daily urine sample, one aliquot was treated with 6 N HCl until urine pH reached 4 determined by a litmus paper test and the second sample was left

untreated. All urine samples were stored in a freezer for later analysis. Fecal bags were removed from each steer and weighed daily during the evening feeding. A 5% aliquot of feces were collected daily and immediately placed in a freezer. Total mixed ration, orts, fecal, and urine samples were analyzed for CP using a LECO FP-2000 analyzer (3000 Lakeview Ave., St. Joseph, MI) to estimate total N balance.

Collections and mineral analysis

Feed samples including TMR and orts were freeze-dried and ground through a 1 mm screen in a Wiley Mill (Thomas Scientific) prior to mineral analysis. All samples excluding urine samples were prepared in a microwave digestion system by adding 10 ml of nitric acid to 0.5g feed and fecal samples. Samples in the 10 ml of nitric acid were covered in Saran^{TM} wrap and left in a ventilated hood over night. The samples were then transported in a pressurized Teflon-lined digestion vessel and placed in the microwave digester under 1200W at 100% power with a 30 min ramp time, max PSI of 180, at 190° C for a 10 min hold time (Gengelback et al, 1994). Post digestion vessels were allowed time to cool for 5 min and then 2 ml of 30% hydrogen peroxide was added to each vessel and let sit unsealed for 15-30 min to allow more time to cool down for handling. Each digested sample was then poured into a separate 25 ml volumetric flask and vessels were rinsed with ddH_2O. The rinsed water was then added to the volumetric flask and additional ddH_2O was included to bring the total volume within the volumetric flasks to 25 ml (CEM Corporation, 1999). Each 200 µl digest and urine sample were pipetted and diluted with 5 ml of a solution containing 0.5% EDTA and Triton X-100, 1% ammonia hydroxide, 2% propanol and 20 ppb of Sc, Rh, In, and Bi as internal standards. Samples were then analyzed for mineral content using an Agilent 7500ce Inductively Coupled Plasma- Mass Spectrometer (ICP-MS). Three modes were used to minimize spectral interferences for mineral analysis. Sulfur and

Cu were analyzed using He-mode and Mo was analyzed in non-gas mode. Urine and water samples were also analyzed for S, Cu, and Mo using an Agilent 7500ce Inductively Coupled Plasma- Mass Spectrometer (ICP-MS). Concentrations of feed, feces, water and urine were determined using an Olympus AU 640e (Olympis America Inc., Center Valley, PA). The Olympus ISE module uses ether membrane electrodes for cations sodium ($\mathbf{Na^+}$) and potassium ($\mathbf{K^+}$) and a molecular oriented PVC membrane electrode for chloride ($\mathbf{Cl^-}$). Specific cations (Na^+, K^+) and anions (Cl^-) develop an electrical potential with ions of interest according to the Nernst Equation. The electrical potential is then compared to the Internal Reference Solution (Block Scientific Inc.) and translated into voltage and then into the ion concentration of the sample.

Water usage by the 12 chambers was measured daily. Daily water consumption per steer was calculated by multiplying daily usage by the rates of individual chamber urine output to total urine output for the 12 chambers. Samples were analyzed for the percent of DM, OM, ADF, NDF, and ADIP. Vessels were hot weighed empty before 1 g samples were added to each vessel and weighed prior to being heated over night in a 105° C oven. Vessels including the sample were then heated in a 500° C oven to determine ash content to calculate OM. Neutral and acid detergent fibers were determined using ANKOM^{220} Fiber Analyzer. Remaining contents from each filter bag that had undergone ADF analysis was then weighed and analyzed for protein content using a LECO analyzer to determine the percent of ADIP.

Statistical analysis

Statistical analysis was performed using PROC MIXED sub-routine of SAS 9.2 (SAS Inst., Inc., Cary, NC). Air flow (cfm) and NOx gas data (ppm) were passed through filters using

SAS 9.2 to eliminate any possible erroneous data that may have been caused from equipment malfunction or from accidental entry of calibration data. Mean output from unfiltered compared to filtered data is provided in the appendices in Table B.1 and Table B.3 for trial 1 and 2, respectively. Air flow that equaled zero and NOx input data greater than 0.35 ppm was filtered out of the data set before analysis. The independent variables or class statements considered for analysis was phase, dietary treatment (**TRT**), date, and chamber. Each model statement included TRT, phase, and the phase by TRT interaction. Date and date by TRT were analyzed separately to determine day effects for NH_3, N_2O, and NOx emissions to help determine the appropriate days to represent mean emission within a steady state. Chamber within treatment was used as the random effect for both phase and day analysis. All nitrogenous gases were analyzed as mass units output, output per NI, and as output per DMI. Mean emissions (NH_3, N_2O, and NOx), DMI, NI, and pH were all tested for normal distribution using Shapiro-Wilk's test and for homogeneous variance using a Levene's test. Ammonia emissions in trial 2 were natural log transformed to satisfy equal variance and normal distribution. Contrast statements were used to determine treatment differences within each phase. Performance data, nitrogen balance, and excreta pH were analyzed using PROC GLM sub-routine of SAS 9.2 (SAS Inst., Inc., Cary, NC) as there was no defined random effect for these analyses. Pair-wise comparisons were analyzed using Tukey-Kramer's test. Statistical analysis on performance data was determined using TRT as the independent variable and average daily gain (**ADG**), DMI, body weight (**BW**), and gain to feed ratio (G:F) as the dependent variables. Nitrogen balance was analyzed similar to performance data only changing independent variables to N in feed, urine, feces, gas, N retained, and percent N retained. Statistical significance was declared at a *P*-value at or below 0.05 and trends at *P*-value at or below 0.10.

RESULTS

Trial 1

In trial 1, cattle tended to gain less weight with the 60% DDGS diet ($P = 0.06$; Table 4.3) compared to cattle on the 40% DDGS diet. Performance data provided in Table 4.3 was determined from the days post arrival up to the day cattle departed the air quality facility. Cattle on all treatments were in positive energy balance and had a rate of gain typical of cattle in metabolism units (Depenbusch et al., 2009; Gunn et al., 2009). Daily weight gain on the 40% and 60% DDGS treatments were similar to control treatments. Dry matter intakes and F:G ratio were similar among treatments. Although not significant, cattle on 60% DDGS diet had numerically lower DMI and G:F than the other treatments. Cattle fed the 60% DDGS diet tended to have lower DM and OM digestibility compared to cattle the fed 60% DDGS diet ($P < 0.10$). The lower performance with the 60% DDGS diet is supported by the low digestibility's observed.

Table 4.3 Effects of dry distiller's grain with soluble on performance in trial 1

	Diet				
Item	Control	40% DDGS[1]	60% DDGS[1]	SEM	*P*- value
Initial BW, kg	252	245	246	22	0.97
Final BW, kg	288	287	274	20	0.84
DMI, kg/d	6.07	6.36	5.74	0.84	0.84
ADG, kg	0.91[ab]	1.06[a]	0.71[b]	0.10	0.06
Gain: feed	0.153	0.183	0.125	0.03	0.28
DM digestibility, %	69.26[ab]	71.35[a]	63.97[b]	2.46	0.10
OM digestibility, %	70.15[ab]	71.30[a]	63.89[b]	2.48	0.09

[1] DDGS- dry distiller's grain with soluble

[a, b] Means without a common superscript within a row differ

Table 4.4 Effects of distiller's grain with soluble levels on nitrogenous emissions for phase 1 and 2 during trial 1

	Phase 1			Phase 2				*P*- value		
Item	0	40	60	0	40	60	SEM	TRT	Phase	TRT × Phase
NH_3, g/d	11.08	10.57	16.85	1.26	2.39	1.49	-	0.88	< 0.01	0.37
*UCL**	*19.34*	*17.13*	*27.30*	*2.20*	*3.07*	*2.42*	-			
*LCL**	*6.35*	*6.52*	*10.40*	*0.72*	*1.48*	*0.92*	-			
NH_3, mg/g NI	95.68	60.96	83.04	16.34	14.31	9.70	12.84	0.55	< 0.01	0.31
NH_3, g/kg DMI	1.90	1.94	2.94	0.41	0.62	0.48	0.44	0.62	< 0.01	0.16
NOx, mg/d†	69.17	60.21	25.31	458.46^{a}	283.48^{b}	311.94^{ab}	49.63	0.15	< 0.01	0.30
NOx, mg/g NI†	0.62	0.37	0.09	3.59^{a}	1.68^{b}	1.34^{b}	0.42	0.03	< 0.01	0.10
NOx, mg/kg DMI	11.39	12.19	4.96	92.97	72.68	62.50	16.04	0.50	< 0.01	0.71
N_2O, g/d	1.85	1.04	1.37	1.70	1.36	1.55	0.31	0.23	0.65	0.74
N_2O, mg/g NI †	16.02^{a}	6.88^{b}	6.19^{b}	12.95	8.37	6.79	2.59	0.06	0.85	0.52
N_2O, g/kg DMI	303.68	195.05	221.85	317.89	373.76	332.14	95.72	0.94	0.18	0.65

[a, b] Means without common superscripts within a phase differ ($P \leq 0.05$)

† Means with superscripts show a linear effect between treatments in a phase ($P \leq 0.05$)

* Upper and lower confidence limits (exponent of untransformed mean ± 1 SEM) express reliability of the estimated mean emissions. Emissions expressed as mass units were back-transformed from a natural log transformation to satisfy normal distribution and equal variance

Phase 1

Mean emissions reported for phase 1 were determined using data from d 11 to d 14 which represent emissions from cattle adjusted to the facility and their assigned diets. Day-to-day variation were highly significant ($P < 0.01$) for NH_3 emissions expressed as mass units per DMI and all units of measure for NOx emissions during phase 1 of trial 1 as shown in appendices (Table B.2). Day by treatment interactions were detected for NOx expressed as mass units ($P = 0.05$) and mass units per DMI ($P = 0.06$). Days selected to represent phase 1 of trial 1 are further illustrated within the appendices in Figures B.1-B.9.

Ammonia and NOx emissions were not different among treatments in phase 1 (Table 4.4). Nitrous oxide emissions expressed as mass units per NI were decreased in the DDGS diets compared to the control diets ($P = 0.02$). Furthermore, N_2O emission expressed as mass units per NI display a linear decrease in emissions as levels of dietary DDGS increase ($P \leq 0.05$). Treatment comparisons are shown graphically in B.10-B.19 in the appendices.

Phase 2

Mean emissions were reported from data collected on the last 2 d of the trial (d 16 and 17) to best report emissions while steers were fitted with fecal bags. Day by treatment interactions were not observed in phase 2 (Figures B.1-B.9). Emissions on d 15 were not used to allow a 1 d adjustment between phase 1 and phase 2. A day effect was reported for NOx emissions when expressed as mass units per DMI during phase 2 (Table B.2).

Ammonia emissions were significantly decreased across all treatments as a result of separating urine and feces in phase 2 ($P < 0.01$; Table 4.4) compared to phase 1. This suggests that most of the NH_3 generated in phase 1 originated from the manure mixture and very little

from enteric fermentation in the animal. In contrast, NOx emissions showed a significant increase during phase 2 compared to phase 1 ($P < 0.01$). The pH and salinity of feces may favor *chemolithotrophic* along with other types of bacteria that oxidize N compounds (Jones and Hood, 1980). Another possible explanation may involve the separation of bacteria in feces and the soluble N compounds in urine which likely limit ureolytic activity (Schmidt et al., 2002). Distiller's grain with soluble diets decreased NOx gas expressed as mass units ($P = 0.03$) during phase 2 compared to the emissions from cattle on the control diet. Emissions from NOx showed a linear decrease ($P = 0.04$) as DDGS in the diet increased. Ammonia and N_2O emissions were similar between treatments for all parameters measured during phase 2.

Feed, feces, and urine collections along with gas data during the last 2 d of trial 1 were used to estimate total N balance by comparing NI from feed to N expelled in the form of feces, urine, and gas. Cattle fed 60% DDGS tended to have a greater feed NI compared to the cattle on the control diet ($P = 0.08$; Table 4.5). Nitrogen intake increased linearly ($P = 0.03$) as levels of DDGS increased in the diet. Expelled N from urine ($P < 0.01$) and feces ($P = 0.09$) showed a linear increase as N in the diet increased. Cattle fed 60% DDGS diets had greater N in urine ($P < 0.01$) as compared to the control. Urine N from cattle fed 40% DDGS diets was intermediate. Nitrogen content in feces and N loss from gas was similar between all diets. Total expelled N was greater in the 60% DDGS diet compared to the control ($P = 0.01$). Total expelled N showed a linear increase as levels of DDGS in the diet increased ($P < 0.01$). Digestible N was greater in the 40 and 60% DDGS diet compared to the control ($P = 0.04$). Similar to NI and N expelled, a linear increase was observed ($P = 0.01$) for N digestibility as DDGS increased in the diet. The amount of N retained on a mass unit basis as well as expressed as a percentage of N intake was similar among treatments. The percent of N retained agrees with other studies as Koeln and

Paterson (1986) reported a N retention from calves fed corn gluten meal (**CGM**), SBM, and toasted soybean meal (**TSBM**) at 44-45% of the NI across all dietary treatments. Additionally, Chen et al. (1977) conducted a study with varying levels of processed distillers soluble and DGS added to diets of beef cattle and reported dietary effects on N balance. Percent N retained from the various diets in this study fell with a range of 25-40%, which are within a similar range of the N retention levels within the current study.

The pH of manure (feces and urine mixture) linearly increased ($P = 0.01$) as the level of dietary DDGS increased (Table 4.6). Manure from cattle on the control diet a lower pH compared to the manure from cattle fed 60% DDGS diet ($P = 0.03$). When pH from feces was measured, there was a tendency ($P = 0.08$) for the control and 60% DDGS diet to be more acidic compared to the 40% DDGS diet. All dietary treatments tended to be more acidic when feces was measured alone compared to the feces and urine mixture. A quadratic effect was present for fecal pH ($P = 0.04$). In contrast to the linear effect on manure pH, urine pH decreased ($P = 0.05$) as the level of dietary DDGS increased. Urine output also linearly increased ($P = 0.05$) as the level DDGS increased in the cattle diets.

Table 4.5 Effects of distiller's grain with soluble on total nitrogen balance for trial 1

	Diet					Contrast	
Item	Control	40% DDGS	60% DDGS	SEM	*P*- value	Linear	Quadratic
N intake, g/d	131.23[a]	207.14[ab]	238.98[b]	30.98	0.08	0.03	0.91
Urinary N, g/d	30.74[a]	59.37[b]	89.61[c]	8.44	< 0.01	< 0.01	0.28
Fecal N, g/d	47.65	53.43	61.52	5.23	0.19	0.09	0.56
N emissions, g/d	3.13	3.95	2.91	1.27	0.82	0.99	0.55
Total N expelled, g/d	81.51[a]	114.95[ab]	154.04[b]	13.22	0.01	< 0.01	0.33
N digestibility[1], %	63.69[a]	74.21[b]	74.26[b]	2.52	0.04	0.01	0.56
N retained, g/d	49.72	92.19	84.94	22.22	0.36	0.22	0.46
N retained, %	28.33	41.98	34.88	5.14	0.55	0.76	0.32

[1] Digestibility calculated by 1- (fecal N/N intake) ×100

[a, b, c] Means without common superscripts within a row differ

* Nitrogen balance was determined from data collected during phase 2 (2 d)

Table 4.6 Effects of feeding distiller's grain with soluble on excreta pH and output during trial 1

	Diet					Contrast	
Item	0% DDGS	40% DDGS	60% DDGS	SEM	*P*- value	Linear	Quadratic
Manure pH[1]	7.73[a]	8.46[ab]	8.76[b]	0.24	0.03	0.01	0.89
Feces pH[2]	6.00[a]	6.64[b]	6.15[a]	0.20	0.08	0.34	0.04
Urine pH[2]	8.67	7.42	6.84	0.62	0.14	0.05	0.97
Dry fecal output[2], kg/d	1.98	1.80	2.11	0.23	0.57	0.82	0.32
Urine output[2], L/d	2.42	5.26	6.69	1.40	0.13	0.05	0.99

[1] Manure- collected during phase 1; mixture of feces and urine

[2] Feces and urine- collected separately during phase 2

[a, b] Treatment means within a row without a common superscript differ

Trial 2

Molybdenum was added in the form of Na_2MoO_4 and Cu was provided through supplemental $CuCl_2$ in the 40% DDGS+ diet. Sodium molybdate and $CuCl_2$ were intended to provide an addition 6 ppm Mo and 60 ppm Cu; however, TMR analyses determined 8 ppm Mo and 90 ppm Cu. The effects of dietary treatments on animal performance are shown in Table 4.7. Cattle were in positive energy balance and consuming more than 2% of their BW. Growth and feed conversion efficiency were similar among treatments. Molybdenum and Cu levels were greater ($P < 0.01$) for the 40% DDGS+ diet. Dry matter digestibility ($P = 0.07$) and OM digestibility ($P = 0.11$) tended to be greater for cattle fed the control diet compared to the cattle fed 40% DDGS+ diet.

Nitrogen emissions were measured as a mass unit output, mass units per unit of NI, and as mass units per unit of DMI. Separation of feces and urine in phase 2 significantly decreased NH_3 emissions ($P < 0.01$; Table 4.8). As shown in trial 1, very little of the NH_3 emitted came directly from the animal but originates from the manure collected in the fecal pans.

Table 4.7 Effects of dry distiller's grain with soluble fortified with copper and molybdenum on performance in trial 2

	Diet				
Item	Control	40% DDGS[1]	40% DDGS+[2]	SEM	*P*- value
Initial BW, kg	300	313	312	16	0.82
Final BW, kg	342	351	339	16	0.86
DMI, kg/d	8.07	7.12	7.49	0.47	0.40
ADG, kg	1.08	0.96	0.70	0.16	0.30
Gain: feed	0.132	0.131	0.091	0.02	0.20
DM digestibility, %	73.90[a]	68.34[ab]	65.49[b]	2.23	0.07
OM digestibility, %	74.41[a]	69.57[ab]	66.66[b]	2.35	0.11
Mo, ppm	1.63[a]	1.38[a]	9.49[b]	0.40	< 0.01
Cu, ppm	12.89[a]	22.29[a]	111.51[b]	4.84	< 0.01

[1] DDGS- dry distiller's grain with soluble

[2] 40 % DDGS+ is supplemented with 8 ppm Mo and 90 ppm Cu

[a, b] Means without a common superscript within a row differ

Table 4.8. Effects of dry distiller's grain with soluble fortified with copper and molybdenum on nitrogenous emissions for phase 1 and 2 during trial 2

	Phase 1			Phase 2				*P*- value		
Item	0	40	40 +	0	40	40+	SEM	TRT	Phase	TRT × Phase
NH_3, g/d	8.56[a]	18.79[ab]	21.23[b]	1.86	1.07	1.34	-	0.83	< 0.01	< 0.01
*UCL**	*11.72*	*25.73*	*29.07*	*2.55*	*1.46*	*0.78*	-			
*LCL**	*6.25*	*13.72*	*15.50*	*1.36*	*1.83*	*0.98*	-			
NH_3, mg/g NI[2]	49.8	87.6	95.5	14.7[a]	5.0[b]	6.4[b]	-	0.69	< 0.01	< 0.01
UCL	*65.5*	*115.2*	*125.6*	*19.0*	*6.5*	*8.4*	-			
LCL	*35.8*	*66.6*	*72.6*	*11.2*	*3.8*	*4.9*	-			
NH_3, g/kg DMI[1]	1.02[a]	2.44[b]	2.58[b]	0.30	0.15	0.19	-	0.90	< 0.01	< 0.01
UCL	*1.36*	*3.26*	*3.45*	*0.40*	*0.20*	*0.25*	-			
LCL	*0.76*	*1.83*	*1.93*	*0.22*	*0.11*	*0.14*	-			
NOx, mg/d	704	476	273	823	785	747	141	0.57	0.97	0.45
NOx, mg/g NI[2]	4.12	2.24	1.54	6.94[a]	3.68[b]	3.73[b]	1.04	0.15	0.21	0.70
NOx, mg/kg DMI	86.0	60.2	39.1	138	109	108	22.9	0.64	0.08	0.97
N_2O, g/d	-2.52[a]	-2.35[ab]	-2.07[b]	-1.83[a]	-1.89[a]	-1.45[b]	0.16	0.05	< 0.01	0.49
N_2O, mg/g NI[1, 2]	-14.6[a]	-11.0[b]	-9.5[b]	-14.1[a]	-8.8[b]	-6.9[b]	0.89	< 0.01	0.13	0.29
N_2O, mg/kg DMI	-303	-311	-254	-283[a]	-268[a]	-200[b]	22.9	0.04	0.15	0.71

[1] 0% DDGS differs from combined 40% DDGS diets in phase 1 ($P \leq 0.05$)

[2] 0% DDGS differs from combined 40% DDGS diets in phase 2 ($P \leq 0.05$)

[a, b] Emissions without a common superscript within a row tend to differ ($P \leq 0.10$)

* Upper and lower confidence limits (exponent of untransformed mean ± 1 SEM) express reliability of the estimated mean emissions

Phase 1

Emissions during trial 2 for phase 1 were determined using a 4 d measurement interval (d 20 to d 23). Day variability was highly significant ($P < 0.01$) for NOx and N_2O emissions but was non-significant for NH_3 emissions (data not shown). Treatment by day interaction was non-significant for all expressed measurements. Daily emission plots for all nitrogenous gases are provided in the appendices (Figures B.19-B.27) with days used for each phase reported. Bar graphs are provided in the appendices for visual comparisons of treatment means within each phase (Figures B.28-B.36).

Addition of DDGS in phase 1 at 40% of the DM increased NH_3 emissions compared to cattle fed the control diet when emissions were expressed as mass units per DMI ($P < 0.05$) as illustrated in Table 4.8. Ammonia emissions expressed as mass units ($P < 0.10$) and mass units per NI ($P = 0.10$) tended to show a similar increase in the 40% DDGS diets compared to the control diet. Conversely, NOx emission tended to decrease in the 40% DDGS diets compared to the control ($P < 0.10$). No differences were determined among treatments when mass units of NOx were adjusted for DMI or NI. Nitrous oxide was significantly lower in the control diet compared to the 40% DDGS diets when mass units were adjusted for NI ($P < 0.01$). Nitrous oxide emissions for both phase 1 and phase 2 generated negative emissions across all treatments. Because of the magnitude of the emissions from zero, these negative emissions may be a result of a detection error for the incoming N_2O gas as chamber emissions are calculated by subtracting the incoming air from the output air of each chamber.

Phase 2

Emissions reported during trial 2 for phase 2 were determined from data collected on d 26 to d 29. Separation of feces from urine in phase 2 decreased NH_3 emissions, whereas NOx and N_2O emissions increased ($P < 0.01$; Table 4.8) compared to phase 1. This inverse relationship may be an affect of decreasing free hydrogen when separating feces from urine, allowing a greater chance for N to undergo oxidation to form NOx gases or N_2O. Ammonia and NOx emissions were greater ($P < 0.05$) in the control diet compared to the 40% DDGS diets when expressed as mass units per NI. Cattle fed 40% DDGS diet fortified with 8 ppm Cu and 90 ppm Mo tended to increase ($P < 0.10$) N_2O emissions compared to 40% DDGS diets without the additional Mo and Cu when emissions were expressed as mass units and mass units per DMI.

Feed, water, feces, and urine collections along with gas data during the last 4 d of trial 2 were used to estimate total N balance by comparing N in feed to N expelled in the form of feces, urine, and gas. Nitrogen loss from gas may be an under-estimate as negative N_2O emissions were included in the calculation. Dietary NI from feed was greater for the 40% DDGS diets compared to NI from steers on the control diet ($P < 0.01$; Table 4.9). Similarly, excreted N in urine ($P < 0.01$) was also greater for both 40% DDGS diets compared to control diets. Nitrogen in gas was emitted at low levels and contributed less than 0.8% and 0.7% of expelled N and N intake, respectively. Total expelled N was greater in both 40% DDGS diets compared to the control ($P = 0.03$). The 40% DDGS+ diet differed from control when comparing mass N retained ($P = 0.03$). The percent of digestible N was greater in the 40% DDGS diets compared to the control diet ($P < 0.01$). This would suggest that N is more available to the animal in the DDGS diets compared to the basal or control diet. The percent of N retained tended to increase in the

40% DDGS diet (P = 0.09) compared to the control and 40% DDGS+ diet. Addition of Cu and Mo to the 40% DDGS diet did not lower N excreted in feces or urine compared to the 40% DDGS diet.

Fecal (P = 0.01) and manure (P = 0.02) pH were greater for both 40% DDGS diets compared to control (Table 4.10). Similar to the results from trial 1, the manure mixture had a greater pH compared to the individual pH of feces or urine.

Table 4.9 Effects of distiller's grain with soluble on total nitrogen balance for trial 2

	Diet				
Item	Control	40% DDGS	40% DDGS+	SEM	*P*- value
N intake, g/d	129.83[a]	215.86[b]	212.40[b]	16.45	< 0.01
Urinary N, g/d	43.15[a]	78.89[b]	77.26[b]	6.72	< 0.01
Fecal N, g/d	62.73	66.40	76.35	5.36	0.23
N emissions, g/d	0.85	-0.01	0.79	0.39	0.27
Total N expelled, g/d	106.72[a]	145.29[b]	154.41[b]	11.32	0.03
N digestibility[1], %	50.52[a]	69.36[b]	63.31[b]	3.22	< 0.01
N retained, g/d	23.11[a]	70.56[b]	57.99[ab]	11.00	0.03
N retained, %	16.85[a]	32.85[b]	26.15[ab]	4.52	0.09

[1] Digestibility calculated by 1- (fecal N/N intake) × 100

[a, b] Means without common superscripts within a row differ

* Nitrogen balance was determined from data collected during phase 2 (4 d)

Table 4.10 Effects of feeding distiller's grain with soluble on excreta pH and output during trial 2

	Diet				
Item	0% DDGS	40% DDGS	40% DDGS+	SEM	*P*- value
Manure pH[1]	7.23[a]	8.21[b]	8.09[b]	0.20	0.01
Feces pH[2]	4.92[a]	5.70[b]	5.56[b]	0.16	0.02
Urine pH[2]	8.05	6.64	6.63	0.52	0.14
Dry fecal output[2], kg/d	2.22	2.29	2.63	0.20	0.35
Urine output[2], L/d	3.81	3.99	4.14	0.73	0.95

[1] Manure- collected during phase 1; mixture of feces and urine

[2] Feces and urine- collected separately during phase 2

[a, b] Treatment means within a row without a common superscript differ

DISCUSSION

Elevated N-emissions from cattle may be influenced by several factors including an increase in urinary N excretion (Erickson et al., 2001) as well as lowering urinary pH (Luebes et al., 1974; Cole et al., 2005). Urine contributes a large majority of its N content in the form of urea. The urea from the urine is hydrolyzed by urease in the feces when mixed, which contributes a large portion of emitted NH_3 (Misselbrook et al., 2005). Within our study, results from both trials show a decrease of NH_3 in phase 2 when feces and urine were collected separately compared to the NH_3 emissions recorded in phase 1. This provides further evidence that feces and urine mixture contribute a large majority of NH_3 emissions. In accord with previous reports (Luebes et al., 1974; Cole et al., 2005), NH_3 emissions increased during phase 1 of both trials as DDGS level and manure pH increased. Acidic urine increases pH when it comes into contact with feces, liberating more NH_3 (Cole et al., 2005; Misselbrook et al., 2005). An inverse relationship seems to exist with NH_3 and NOx emissions, as emitted NOx increased in phase 2 compared to phase 1 in both trials. A study measuring N-emissions from coal combustion reported that an increase in NH_3 production decreased the conversion of fuel N to NOx (Kambara et al., 1995). An increase of volatile N may limit the N available to undergo oxidation. Emissions from NOx also decreased as dietary N increased from greater levels of DDGS in the diet. In trial 1, NOx and N_2O linearly decreased as levels of dietary DDGS increased. In trial 2, 40% DDGS diets emitted greater levels of NH_3 for all parameters measured compared to the control diets. Nitrous oxide levels decreased as the DDGS level increased in

trial 1when expressed as mass units per NI, while the opposite seemed to occur in trial 2. However, since estimates were negative in trial 2 the reader should be cautious of the results.

In trial 1, NI linearly increased as the level of dietary DDGS increased ($P = 0.03$). Urinary N ($P < 0.01$), fecal N ($P = 0.09$), and total N expelled ($P < 0.01$) all linearly increased as levels of feed N increased. Digestible N was greater in the DDGS diets compared to the control diet as shown in both trials. These results are surprising as DGS is reported to offer a greater fraction of ruminally undegradable intake protein (**UIP**) and lower degradable intake protein (**DIP**) in cattle diets (Bothast and Schlicher, 2005). However, a study from Salisbury et al. (2004) reported no differences in N digestibility and N retention in wethers fed low and high levels of UIP. The percent of N retained from both trials show a greater N utilization in DDGS diets compared to the control diet. Fron et al. (1990) reported that cattle diets containing greater levels of sulfur (**S**) tend to utilize dietary N more efficiently. The present study supports the literature as dietary S intake increased from approximately 13 g/d in the control diet to 35 and 45 g/d in the 40 and 60% DDGS diets, respectively (data not shown).

In trial 2, the dietary treatment of 40% DDGS+, contained 8 ppm Mo and 90 ppm Cu. The purpose of this treatment was to mitigate the amount of available S in the cattle's diet that could be reduced by sulfate-reducing bacteria (**SRB**) in the rumen and manure. However, limiting available S in ruminant diets may indirectly influence N-emissions. Bouchard and Conrad (1972) reported that a N:S ratio exists; as dietary S decreases so would the percent of N retained, causing an increase in excreted N (Rumsey 1978). Based on the results from trial 2, NH_3 emissions tended to show a linear increase from the control, 40% DDGS, and 40% DDGS+ diet for all parameters measured ($P \leq 0.10$). Averaging dietary treatments in phase 1, NH_3 gas contributed 90% of the total N-emissions.

The collection system used during phase 1 of each trial more accurately portrays emissions from liquid manure handling and storage systems. Manure (feces and urine) mixture during phase 1 was the main source of NH_3 emissions recorded within the chamber rooms. As mentioned previously, excreta pH (Luebes et al., 1974; Cole et al., 2005) and mixture of urine and feces (Misselbrook et al., 2005) contribute to an increase in NH_3 emissions. During trial 2 of the current study, an increase in NH_3 emissions did tend to correlate to diets that produced greater manure pH. Distiller's grain with soluble diets had increased levels of dietary S and it has been shown that urine pH will decrease with high sulfide contents (Thistlethwayte, 1972; Xue et al., 1998). Conversely, manure pH increases as reduced forms of S and N react with free hydrogen and are emitted from the manure as H_2S and NH_3, respectively. Results from both trials agrees with previous reports (Thistlethwayte, 1972; Xue et al., 1998) by offering further evidence that an increase in manure pH will emitt greater levels of NH_3 along with H_2S from the manure.

Feeding DDGS at 60% of the diet DM did not contribute to elevated N-emissions compared to 40% DDGS diets despite increasing urinary N excretion and decreasing urinary pH. Continued research would be beneficial to provide a better understanding of the relationship of available S in the diet with N retention and N-emissions. Furthermore, other studies suggest that reducing CP in the diet by at least 5 percentage units in the diet composition can drastically reduce total N excretion by 30% (Misselbrook et al., 2005) to 66% (Castillo et al., 2000). Our data falls within the range of the results from Misselbrook et al. (2005) and Castillo et al. (2000) as the dietary CP was decreased from 24.6 (60 % DDGS) to 12.9 % of the dietary DM (control), N excretion was decreased 47%. The current study suggests adding DDGS to cattle diets does

contribue to an increase in NH_3 emissions compared to traditional corn-base diets (control).

Because of the growing availability to cattle producers along with lower costs to feed to cattle as an energy and protein source, it may be advantageous to determine a strategy that could mitigate N loss as gas. Furthermore, manure contains plant available N that can be used as a fertilizer for corn production to improve soil quality and corn yield (Sawyer, 2001). Therefore, manure high in N may provide an aconomic incentive for use as a fertilizer for corn producers. Further research should be conducted to determine a strategy to decrease N-emissions from manure associated with cattle being fed diets containing high levels of CP, particularly with dry and wet forms of DGS.

CHAPTER 5: CARBON FOOTPRINT AND ENERGY PARTITION FROM CATTLE FED VARIOUS LEVELS OF DISTILLER'S GRAIN WITH SOLUBLE

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SUMMARY

A study was conducted to evaluate the effects of DDGS on C-containing gases, GHG footprint, and energy retention. Two trials were conducted within this study. Both trials were conducted with 12 Holstein steers housed in individual environmentally-controlled rooms to monitor gas production. Three dietary treatments were fed in trial 1; containing 0% (control), 40%, and 60% DDGS. In trial 2, treatments were the same except the 60% DDGS dietary treatment was replaced with a 40% DDGS diet fortified with 8 ppm Mo and 90 ppm copper Cu (40% DDGS+). Each trial was divided into two phases; phase 1 of each trial monitored emissions when urine and feces were collected in the same vessel. Phase 2 of trial 1 monitored emissions for 2 d while phase 2 of trial 2 monitored emissions for 4 d while steers were fitted with fecal bags to separate feces from urine. In trial 1, the 40% DDGS diet emitted 23% less GHG (N_2O, CH_4, and CO_2) compared to the control diets. In trial 2, the 40% DDGS+ treatment decreased CO_2 emissions ($P \leq 0.05$) and tended to also reduce CH_4 emissions ($P = 0.08$) compared to the control and the traditional 40% DDGS diet. An energy balance was calculated from phase 2 of each trial. In trial 1, UE linearly increased as the level of DDGS increased ($P = 0.02$). In trial 2, the 40% DDGS diets had a greater retained energy or NEg compared to the control ($P = 0.02$).

Key words: distiller's grain with soluble, methane, carbon dioxide, greenhouse gas, energy

INTRODUCTION

Distiller's grain with soluble (**DGS**) is nearly 3-fold more concentrated in protein, fat, fiber, and other minerals (Stock et al., 2000). This increase in nutrient concentration in DGS is the result of removing the starch from the corn kernel during the dry milling process for ethanol production (Klopfenstein et al., 2008). The availability and cost benefits of feeding DGS have made it a very popular energy and protein replacement over the traditional use of corn and soybean meal (**SBM**) in diets. A fraction of the dietary gross intake energy (**IE**) is lost as methane (**CH_4**) gas in cattle due to fermentation of carbohydrates by ruminal methanogens. Methane production typically accounts for 5.5 to 6.5% of the IE (Johnson and Ward, 1996; Kurihara et al., 1998), but has been reported to account for as high as 12% of the loss from feed IE (Behlke et al., 2007). Aside from CH_4 production accounting for energy loss, it is also a potent greenhouse gas and has been associated with global warming. Of the total CH_4 emitted from all sources each year, 58 million tons/yr or 73% was contributed by livestock species (US Environmental Protection Agency, 1994). In a more recent report, livestock contributed about 40% of the total CH_4 (FAO, 2006). Based on the unit of dry matter intake (**DMI**), forage-based diets produce more CH_4 than cereal grain diets when fed to ruminant animals (Behlke et al., 2007). However, limited research has been conducted to determine available energy from dry distiller's grain with soluble (**DDGS**) and what impact feeding DDGS to feedlot cattle may have on CH_4 production. Two trials were conducted; the objective of the first trial was to address this issue by determining if increasing dietary DDGS would affect CH_4 emissions and the total greenhouse gas (**GHG**) footprint, along with the level of retained or net energy (**NEg**) available

to the animal. In trial 2, supplemental copper (**Cu**) and molybdenum (**Mo**) were included to mitigate S-containing emissions at 90 and 8 ppm, respectively. However, it was also important to determine what affects additional Cu and Mo may have on performance, energy retention, and CH_4 production.

MATERIALS AND METHODS

Gas collection

Two studies were conducted at Michigan State University at the Animal Air Quality Research Facility (**AAQRF**) to address concerns associated with high inclusion levels of DDGS. Approval for this study was provided by the Michigan State University Animal Care and Use Committee (AUF # 07/09-110-00). Environmentally-controlled rooms at AAQRF monitor incoming ambient and outgoing air from each of the 12 rooms for concentrations of CH_4, non-methane volatile organic compounds (**VOCs**), nitrous oxide (**N_2O**), as well as respired carbon dioxide (**CO_2**) and oxygen (**O_2**) consumption. Before arrival to AAQRF, steers were held at Michigan State University's Beef Cattle Teaching and Research Center (**BCTRC**) where they were weighed and vaccinated for prevention of clostridial disease using Ultrabac-7 (Pfizer, New York, NY) and respiratory infections (Bovi-Shield GOLD® 5 Pfizer, New York, NY). At trial initiation; a 2 wk adjustment time was allotted at BCTRC before steers were transported to AAQRF. During this time, all 12 steers were started on a corn-based concentrate diet (control diet). After the first week, 8 steers were randomly assigned and adjusted to a 40% DDGS diet. The control diet consisted of 81% high moisture corn (**HMC**), 10% corn silage (**CS**), 5% SBM, and 4% mineral supplement. The 40% DDGS diet consisted of 40% DDGS, 46% HMC, 10% CS, and 4% mineral supplement. Steers were broke to lead to ensure safe handling and limit stress on the animal during the study at AAQRF. Four days prior to arrival at AAQRF, steers were housed in metabolism stalls at BCTRC to allow adaptation to similar living conditions that they would have in the individual, environmentally-controlled rooms at AAQRF. Steer weights were taken before feeding on 2 consecutive days before transportation to AAQRF. Weights

were also taken on 2 consecutive days upon return to the feedlot facility. Steers (n=12) were randomly assigned to three dietary treatments prior to arrival at AAQRF. In trial 1, 4 steers from the 40% DDGS diet on d 11 of being housed in environmentally controlled rooms were randomly switched to a 60% DDGS diet.

Two different trials were conducted at AAQRF to determine the effects of increasing DDGS on animal performance, C-containing emissions and total greenhouse footprint, and energy retention. The dietary treatments for trials 1 and 2 are shown in Table 5.1. In trial 1, 3 levels of DDGS were fed (0, 40, and 60%) and 2 levels in trial 2 (0 and 40%) with 4 steers per treatment. In trial 2, the third treatment was 40% DDGS fortified with 8 ppm Mo and 90 ppm Cu. The latter treatment was called 40% DDGS+. The source of Mo was sodium molybdate (**Na_2MoO_4**) and the Cu source was copper chloride (**$CuCl_2$**). Copper chloride was selected to minimize the amount of additional S in the diet. All diets during trial 1 were top dressed daily with thiamine (200 mg) as a preventive step against S toxicity. Eye and ear twitching was recorded daily to monitor physical symptoms associated with PEM. Recorded twitches for each steer were unchanged during both studies. On d 5 of trial 1, a steer on the control diet had to be pulled off the study due to poor feed intake and formation of hematoma near the left hook bone.

Table 5.1 Dietary ingredients and composition for trial 1 and 2

	Trial 1			Trial 2		
Ingredient, % of DM	Control	40	60	Control	40	40 +[2]
DDGS[1]	-	40	60	-	40	60
High moisture corn	81	46	26	81	46	26
Corn silage	10	10	10	10	10	10
Soybean meal	5	-	-	5	-	-
DGS supplement	-	-	-	-	4	4
BFS50 supplement	4	4	4	4	-	-
Total	100	100	100	100	100	100
			Diet composition			
Dry matter, %	67.7	74.8	76.0	68.6	73.6	74.5
			% of DM			
Ash	5.6	7.4	8.0	4.4	6.4	7.1
ADF	5.9	7.1	8.8	4.2	7.2	7.0
NDF	11.8	19.0	23.2	11.7	19.9	19.6
Starch	60.3	33.5	22.1	58.6	33.5	33.3
Ether extract	2.9	4.6	5.5	2.2	3.9	3.9
Crude protein	12.9	21.5	24.6	12.9	19.1	18.8
ADIP[3]	1.5	5.5	6.6	1.3	4.5	5.0
Gross energy, Mcal/kg	4.0	4.3	4.4	4.4	4.7	4.7

Ingredients, % of DM	BFS50 supplement	DGS supplement
Akey TM premix # 4*	1.4	2.4
Limestone	24.9	71.5
Soybean meal, 48% N	48.3	-
Rumensin[TM] 80	0.3	0.4
TM salt	9.6	18.0
Vitamin E, 5%	0.2	0.1
Urea, 45% N	9.6	7.6
Potassium chloride	5.1	-
Selenium 90	0.7	-
Total	100	100

[1] DDGS- dry distiller's grain with soluble

[2] 40 + is fortified with supplemental 8 ppm molybdenum and 90 ppm copper

[3] ADIP- acid detergent insoluble proteins

* Akey TM premix # 4 composition: 9% Mg, 4% S, 0.02% Co, 1% Cu, 0.09% I, 2% Fe, 4% Mn, 0.03% Se, 4% Zn, 4,400,000 IU vitamin A, 550,000 IU vitamin D, and 5,500 IU vitamin E/kg (Akey Inc., Lewisburg, OH)

Steers were adapted to diets and chamber rooms for 10 d (trial 1) prior to collecting emissions data and1 d in trial 2. Emissions were recorded over 14 d in trial 1 and 22 d in trial 2. Emissions data from the last 4 d were summarized and reported as emissions during phase 1. Temperature in the chambers was maintained near 16° C and air flow was closely regulated near 17 m^3/min or 40 air changes per hour. Humidity in each chamber room was recorded but was not regulated. Sampling cycle of the 12 rooms plus the background air would take 3 h 15 min, which allowed 7 to 8 observations per room per d. During the 15 min sampling cycle, the first 9.5 min were used as a purging period and gaseous content of air was recorded the last 5.5 min. Exhausted air was sampled within an aluminum duct in each room where Teflon coated sample lines would draw air to the gas analyzers by positive static pressure from each individually sealed chamber room. Each room was also fitted with an in-house manufactured type-J thermocouple, and a Campbell Scientific ™ HMP45C temperature and relative humidity probe (Logan, Utah, Campbell Scientific, Inc.). Methane and VOCs were measured using a TEI 55C Direct Methane, Non-methane Hydrocarbon Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) by back-flushed chromatography. Carbon dioxide and O_2 were measured using a BINOS® 100 2M Dual-Channel Gas Analyzer (Rosemount Analytical Inc., Orrville, OH, USA) by applying a combination of infrared sensors, thermo conductivity, electrochemical and paramagnetic oxygen sensors. Gas sampling and data collection were all computer controlled by LabView 8.2.1 software and the FieldPoint system (National Instruments, Austin, TX, USA). Analyzers output concentration was measured in ppm and was transposed into units of mg/min. The units were then adjusted to 24 h and expressed as mass unit output, output adjusted for DMI, and output adjusted for DE intake.

Feed, feces and urine collection

During each gas collection period for trial 1 and trial 2, daily samples of total mixed ration (**TMR**) and feed weigh-back (**orts**) from each steer were collected and immediately placed in a freezer for storage. Orts were monitored daily to adjust the amount of TMR offered to 110% of the previous d delivery. All feed samples and orts were later freeze-dried and ground through a Wiley Mill (Thomas Scientific) fitted with a 2 mm screen followed by a 1 mm screen. Stalls were set on an incline to allow urine to flow back and be collected in the manure pan as well as excreted feces. The feces and urine mixture was maintained at a 5 cm depth inside the pan during phase 1 of each trial. Partial removal of excreta was performed daily to maintain a 5 cm depth in the pan. The removed excreta were weighed to calculate total excretion. A 0.5 kg sample of excreta was collected during each removal and immediately stored in a freezer for later analysis. Excreta removal was collected during the morning shift, whereas feeding along with TMR and ort collections were conducted in the afternoon. During each shift, chamber rooms and stalls were thoroughly cleaned and chamber rooms were only entered if they were 5 chambers or more ahead of gas sampling.

Feces and urine segregation using fecal bags

On d 15 of trial 1 and d 23 of trial 2, all manure and urine was removed and weighed from each fecal pan. Fecal bags were placed on each steer and clean pans were put back in place to collect urine only. Fecal and urine segregation took place during the last 2 d of trial 1 and the last 4 d of trial 2. This period of each trial will be referred to as phase 2. Urine pans were pulled twice daily and filtered through a screen before urine weight and volume were recorded. A 2% aliquot was collected daily of the composited urine sample. All urine samples were stored in a freezer for later analysis. Combined water usage of the 12 chambers was measured daily. Daily

water consumption per steer was calculated by multiplying daily total usage by the rates of individual chamber urine output to total urine output for the 12 chambers. Fecal bags were removed from each steer and weighed daily during the evening feeding. A 5% aliquot of feces were collected daily and immediately placed in a freezer. Total mixed ration, orts, fecal, and urine samples were analyzed for IE using a Parr Oxygen Bomb Calorimeter (Parr Instrument Company, Moline, IL, USA) to estimate total energy partition for each diet within trial 1 and trial 2. Urine samples were freeze-dried and the DM fraction was used to calculate urine energy.

Energy balance calculations

Energy values expressed in MJ/d for TMR, orts, feces, and urine were determined from samples collected during phase 2 of trial 1 and 2 when feces and urine were collected separately. Gross intake energy (IE) was determined by subtracting the ort's total energy from the total energy offered within the TMR for each steer. The total loss is the sum of energy loss in feces, urine, and CH_4 gas. Digestible energy (**DE**) was calculated as the difference of fecal energy (**FE**) from IE. Metabolizable energy (ME) was calculated by subtracting urine (**UE**) and CH_4 gaseous energy (**GE**) from DE. Energy from CH_4 gas was calculated by using conversion factors 39.54 KJ/L and 0.716 g/L (Brouwer, 1965; Kurihara et al., 1998). Heat energy (**HE**) was estimated using the equation: HE = 16.26 KJ × O_2 (L/d) + 5.02 KJ × CO_2 (L/d) – 2.17 KJ × CH_4 (L/d) – 5.99 KJ × N (g/d) (Brouwer, 1965; Ferrell et al., 1988; Kurihara et al., 1998). Methane emissions were analyzed as g/d and was converted to L/d by dividing the gases mass output (g/d) by its molecular weight (g/mol) and then multiplying the conversion factor 22.42 (L/mol). Net energy (NEg) represents the difference of HE from ME.

Collections and mineral analyses

Feed samples including TMR and orts were freeze-dried and ground through a 1 mm screen in a Wiley Mill (Thomas Scientific) prior to mineral analysis. All samples excluding urine samples were prepared in a microwave digestion system by adding 10 ml of nitric acid to 0.5g feed and fecal samples. Samples in the 10 ml of nitric acid were covered in Saran^{TM} wrap and left in a ventilated hood over night. The samples were then transported in a pressurized Teflon-lined digestion vessel and placed in the microwave digester under 1200W at 100% power with a 30 min ramp time, max PSI of 180, at 190° C for a 10 min hold time (Gengelback et al, 1994). Post digestion vessels were allowed time to cool for 5 min and then 2 ml of 30% hydrogen peroxide was added to each vessel and let sit unsealed for 15-30 min to allow more time to cool down for handling. Each digested sample was then poured into a separate 25 ml volumetric flask and vessels were rinsed with ddH_2O. The rinsed water was then added to the volumetric flask and additional ddH_2O was included to bring the total volume within the volumetric flasks to 25 ml (CEM Corporation, 1999). Each 200 µl digest and urine sample were pipette and diluted with 5 ml of a solution containing 0.5% EDTA and Triton X-100, 1% ammonia hydroxide, 2% propanol and 20 ppb of Sc, Rh, In, and Bi as internal standards. Samples were then analyzed for mineral content using an Agilent 7500ce Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). Three modes were used to minimize spectral interferences for mineral analysis. Sulfur and Cu were analyzed using He-mode and Mo was analyzed in non-gas mode. Urine and water samples were also analyzed for S, Cu, and Mo using an Agilent 7500ce Inductively Coupled Plasma- Mass Spectrometer (ICP-MS). Concentrations of feed, feces, water and urine were determined using an Olympus AU 640e (Olympis America Inc., Center Valley, PA). The

Olympus ISE module uses ether membrane electrodes for cations sodium ($\mathbf{Na}^{+}$) and potassium ($\mathbf{K}^{+}$) and a molecular oriented PVC membrane electrode for chloride ($\mathbf{Cl}^{-}$). Specific cations (Na^{+}, K^{+}) and anions (Cl^{-}) develop an electrical potential with ions of interest according to the Nernst Equation. The electrical potential is then compared to the Internal Reference Solution (Block Scientific Inc.) and translated into voltage and then into the ion concentration of the sample.

Feed, fecal and urine samples were analyzed for the percent of DM, OM, ADF, NDF, starch, ether extract, CP, ADIP, and gross energy. Vessels were hot weighed empty before 1 g samples were added to each vessel and weighed prior to being heated over night in a 105° C oven for DM determination. Vessels including the sample were then placed in a 500° C oven to determine ash content to calculate OM. Neutral and acid detergent fibers were determined using ANKOM^{220} Fiber Analyzer (Ankom Technology, Macedon, NY) following the procedure outlined by Ankom Technology (1998). Remaining contents from each filter bag that had undergone ADF analysis was then weighed and analyzed for protein content using a LECO FP-2000 analyzer (Leco Corp., St. Joesph, MI) to determine the percent of ADIP. Crude protein was also determined on feed, fecal, and urine samples using a LECO analyzer. Ether extractable fat was determined with a modified soxhlet extraction procedure (Association of Analytical Chemists, 1990). Starch and free sugar analysis using a plate reader (Karkalas, 1985) was performed following Karkalas (1985) procedure.

Statistical analysis

Statistical analysis was performed using PROC MIXED sub-routine of SAS 9.2 (SAS Inst., Inc., Cary, NC). Air flow (cfm), CO_2 (ppm) and O_2 (%) were passed through filters using

SAS 9.2 to eliminate any possible erroneous data that may have been caused from equipment malfunction or from accidental entry of calibration data. Mean output from unfiltered compared to filtered data is provided in the appendices in Table A.1. Air flow that equaled zero, CO_2 greater than 1200 ppm, and O_2 greater than 21.4% was filtered out of the data set before analysis. The independent variables or class statements considered for emissions analysis were phase, dietary treatment (TRT), date, and chamber. Each model statement included TRT, phase, and the phase by TRT interaction. Date and date by TRT were analyzed separately to determine day effects for CH_4, VOC, CO_2, and O_2 emissions to help determine the appropriate days to represent mean emission within a steady state. Chamber within treatment was used as the random effect for both phase and day analysis. Methane, VOC, and CO_2 gases were analyzed as output mass units per d, output per kg of DMI, and as output per Mcal of DE. Carbon dioxide, CH_4, and N_2O were adjusted for global warming potential (GWP) and analyzed using the same PROC MIXED procedure as the other dependent emission variables. Mean emissions were all tested for normal distribution using Shapiro-Wilk's test and for homogeneous variance using a Levene's test. Contrast statements were designed for pair-wise treatment comparison, along with linear and quadratic contrasts in trial 1. In trial 2, orthogonal comparisons of the control vs. the 2 DDGS diets were considered.

Performance data and energy partition were analyzed using PROC GLM sub-routine of SAS 9.2 (SAS Inst., Inc., Cary, NC) as there was no defined random effect for these analyses. Pair-wise comparisons were analyzed using Tukey-Kramer's test. Statistical analysis on performance data was determined using TRT as the independent variable and average daily gain (**ADG**), DMI, body weight (**BW**), and gain to feed ratio (**G:F**) as the dependent variables.

Energy partition was analyzed similar to performance data only changing independent variables to IE, FE, UE, GE, total energy loss, DE, ME, HE, and energy retention or NEg. Statistical significance was declared at a *P*-value at or below 0.05 and trends at *P*-value at or below 0.10.

RESULTS

Trial 1

In trial 1, cattle tended to gain less weight per d when fed the 60% DDGS diet (P = 0.06; Table 4.2) compared to cattle on the 40% DDGS diet. Daily weight gain for cattle fed the control diet was similar to both 40 and 60% DDGS diets. Dry matter intakes and G:F were similar among treatments. Cattle fed the 60% DDGS diet had numerically lower DMI and G:F than cattle fed the other treatments. Cattle fed the 40% DDGS diet tended to have greater digestibility compared to cattle the fed 60% DDGS diet ($P = 0.10$). Performance data provided in Table 4.2 was determined from the time period between entry and departure from AAQRF (22d). Cattle fed all treatments were in positive energy balance and had a rate of gain typical of cattle in metabolism units (Depenbusch et al., 2009; Gunn et al., 2009).

Table 5.2 Effects of dry distiller's grain with soluble on performance in trial 1

	Diet				
Items	Control	40% DDGS[1]	60% DDGS	SEM	*P*- value
Initial BW, kg	252	245	246	22	0.97
Final BW, kg	288	287	274	20	0.84
DMI, kg/d	6.07	6.36	5.74	0.84	0.84
ADG, kg	0.91[ab]	1.06[a]	0.71[b]	0.10	0.06
Gain: feed	0.153	0.183	0.125	0.03	0.28
DM digestibility, %	69.26[ab]	71.35[a]	63.97[b]	2.46	0.10
OM digestibility, %	70.15[ab]	71.30[a]	63.89[b]	2.48	0.09

[1] DDGS- dry distiller's grain with soluble

[a, b] Means without a common superscript within a row differ ($P \leq 0.10$)

Table 5.3 Effects of distiller's grain with soluble levels on carbon emissions and respiration for phase 1 and 2 during trial 1

	Methane emissions									
	Phase 1			Phase 2				*P*- value		
Item	0	40	60	0	40	60	SEM	TRT	Phase	Phase × TRT
g/d	45.12	32.91	42.60	43.22	31.30	39.68	6.30	0.34	0.50	0.98
g/kg DMI	7.33	5.13	6.91	8.58	6.76	8.73	1.08	2.28	0.04	0.92
g/Mcal DE	3.63	2.88	3.37	3.46	2.47	3.12	0.54	0.47	0.37	0.94
	Non-methane VOC emissions									
g/d	2.27	2.00	1.64	0.69	0.75	0.41	0.28	0.45	< 0.01	0.22
mg/kg DMI†	361.94[a]	306.55[ab]	264.09[b]	140.97	143.98	95.06	35.71	0.27	< 0.01	0.47
mg/Mcal DE†	126.02[a]	116.44[ab]	88.36[b]	38.31	38.05	22.46	13.29	0.24	< 0.01	0.46
	Respiration									
O_2, kg/d	-6.39	-6.10	-6.40	-4.51	-4.37	-4.80	0.78	0.94	< 0.01	0.76
CO_2, kg/d	4.42	3.58	3.80	3.96	3.44	3.46	0.37	0.41	< 0.01	0.33
CO_2, g/kg DMI	715	568	611	779	822	760	100	0.85	0.03	0.47
CO_2, g/Mcal DE	246	220	208	220	211	190	36.3	0.78	< 0.01	0.40

[a, b] Means without common superscripts within a row for each phase differ ($P \leq 0.10$)

† Means within a phase tend to show a linear effect ($P \leq 0.10$)

Phase 1

Emission data from d 11 to 14 were selected to represent mean emissions at a stable condition for phase 1 (Table 5.3). During phase 1, only O_2 emissions ($P = 0.02$) and CO_2 adjusted for DMI ($P = 0.05$) had a significant day effect (data not shown). Methane and respiratory gases (CO_2 and O_2) were similar among treatments during phase 1. Non-methane VOC emissions tended to show a linear reduction in emissions as DDGS increased in the diet ($P < 0.10$) when adjusted for DMI or DE (Table 5.3).

The total GHG footprint was estimated using a global warming potential (**GWP**) factor fixed over a 100 yr period (Table 4.4). Cattle fed the 40% DDGS diet tended to have the lowest GHG footprint compared to the control and 60% DDGS diets, however differences were non-significant. The 3 major GHGs recorded were; CO_2, which contributes around 66-70%, CH_4 accounted for 23-27%, and N_2O 6-8% gram equivalents. According to Lashof and Ahuja (1990), CO_2 makes up approximately 70% of the total gases that contribute to the GWP within the atmosphere. This is similar to the percent emitted from the cattle within the current study.

Phase 2

Mean emissions that represent phase 2 were selected the last 2 d of the trial (d 16 and 17) to allow a 1 d adjustment before reporting emissions data while cattle were fitted with fecal bags. Methane adjusted for DMI ($P < 0.01$), both measures for non-methane VOC ($P < 0.01$), CO_2 adjusted for DMI ($P = 0.03$), and O_2 ($P < 0.01$) had significant day effects (data not shown). All of the gases that showed d variability had similar 2 d averages. The differences between days suggest that more than a 2 d collection period is needed. Separation of feces and urine in the

chamber rooms did not affect CH_4 emissions mass output when comparing phase 1 and 2. However, phase 2 emissions were elevated compared to phase 1 when CH_4 was adjusted for DMI ($P = 0.04$) due to a reduction in DMI when cattle were fitted with fecal bags during phase 2. All parameters of non-methane VOC emissions were significantly decreased in phase 2 compared to phase 1 ($P < 0.01$). Oxygen consumption and emitted CO_2 also declined during phase 2 compared to phase 1 ($P < 0.01$).

The estimated GHG footprint for all dietary treatments during phase 2 was lower ($P < 0.01$) than phase 1, which can be attributed to less CO_2 emissions (Table 5.4). Carbon dioxide was emitted within a range of 64-69% of the total GHG footprint. The contribution to the total GHG from CH_4 and N_2O were similar to the levels indicated from phase 1, producing 22-27% and 8-9%, respectively.

Gross intake energy (IE) was similar among dietary treatments within phase 2 during trial 1 (Table 5.5). Energy loss in urine linearly increased as inclusion levels of DDGS increased in the diet ($P = 0.02$). This is likely the result of spillage of N compounds into the urine as the level of DDGS increased. However, no treatment differences were determined with FE, GE, and total energy loss. Digestible energy, ME, HE, and NEg were similar among dietary treatments as well. Based on the NE values from trial 1 at 0, 40, and 60% DDGS diets; predicted values were determined for 80 and 100% DDGS (Figure 5.1). A polynomial line equation was determined using the actual values (0, 40, and 60% DDGS) to generate the predicted NEg for 80 and 100% DDGS.

Table 5.4 Global warming potential gram equivalents per day from greenhouse gas emissions from cattle fed various levels of distiller's grain with soluble during trial 1. Values in parentheses are percentages of the total

		Phase 1					Contrast	
Item	GWP_{100} [1]	Control	40 % DDGS	60% DDGS	SEM	*P*- value	Linear	Quadratic
CO_2***	1	4417.48 (67.0)	3575.51 (70.5)	3801.90 (66.2)	373.44	0.31	0.19	0.37
CH_4	25	1624.14 (24.6)	1184.91 (23.4)	1533.46 (26.7)	226.90	0.39	0.58	0.21
N_2O	298	552.70 (8.4)	310.14 (6.1)	407.87 (7.1)	91.39	0.24	0.19	0.23
Total	-	6594.32 (100)	5070.56 (100)	5743.23 (100)	456.13	0.19	0.17	0.17
		Phase 2						
CO_2	1	3961.93 (65.8)	3438.77 (69.2)	3464.90 (64.7)	373.44	0.55	0.31	0.68
CH_4	25	1555.95 (25.8)	1126.88 (22.7)	1428.31 (26.7)	226.90	0.43	0.52	0.25
N_2O	298	507.78 (8.4)	404.18 (8.1)	461.74 (8.6)	91.39	0.73	0.63	0.53
Total	-	6025.66 (100)	4969.83 (100)	5354.94 (100)	456.13	0.40	0.29	0.37

[1] GWP_{100} indicates global warming potential within a fixed 100 yr period from the Intergovernmental Panel on Climate Change (2007)

[a, b] Means without common superscripts within row differ

*** Phase 1 differs from phase 2 ($P < 0.01$)

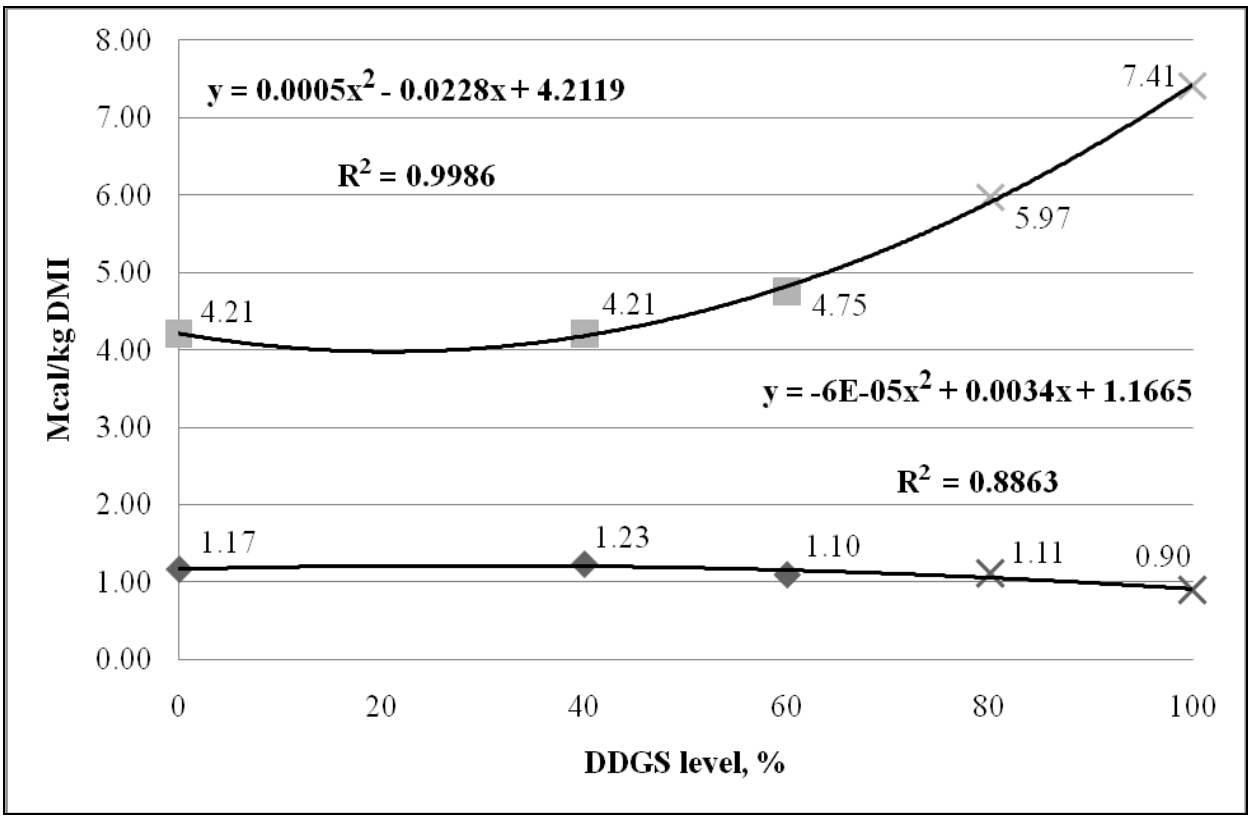
Table 5.5 Effects of distiller's grain with soluble on energy partition during trial 1 (MJ/d)

	Diet					Contrast	
Item	Control	40% DDGS	60% DDGS	SEM	*P*- value	Linear	Quadratic
Intake energy	107.31	112.33	114.36	14.84	0.94	0.73	0.99
Feces	32.36	34.15	36.68	4.21	0.74	0.49	0.84
Urine	2.04[a]	3.23[ab]	4.08[b]	0.48	0.04	0.02	0.79
Methane	2.39	1.73	2.19	0.36	0.45	0.53	0.26
Total loss	36.78	39.11	42.95	4.48	0.64	0.40	0.77
Digestible energy	74.95	78.18	77.67	11.63	0.98	0.85	0.92
Metabolizable energy	70.53	73.21	68.00	11.08	0.98	0.93	0.88
Heat energy[1]	40.93	40.47	45.11	8.83	0.91	0.77	0.77
Net energy (gain)	29.60	32.75	26.30	9.91	0.89	0.87	0.67

[1] HE = 16.26 KJ × O_2 (L/d) + 5.02 KJ × CO_2 (L/d) – 2.17 KJ × CH_4 (L/d) – 5.99 KJ × N (g/d); Brouwer (1965)

[a, b] Means within a row without common superscripts differ

Figure 5.1 Predicted energy retention from cattle fed dried distiller's grain with soluble estimated from trial 1 results



♦ = actual measured values for NEg

■ = actual measured values for IE

× = predicted values

Trial 2

Molybdenum was added in the form of Na_2MoO_4 and Cu was provided through supplemental $CuCl_2$ in the 40% DDGS+ diets. Sodium molybdate and $CuCl_2$ were included to provide additional Mo and Cu. The actual intake of Mo and Cu to the 40% DDGS+ diet was 8 and 90 ppm, respectively. The effects of dietary treatments on animal performance are shown in Table 5.6. Cattle were in positive energy balance and consuming more than 2% of their BW. Growth and feed conversion efficiency were similar among treatments. Dry matter digestibility ($P = 0.07$) tended to be greater in cattle fed the control diet compared to the cattle fed 40% DDGS+ diet. Dietary levels of Mo and Cu were greater ($P < 0.01$) for the DDGS+ diet compared to the control and traditonal 40% DDGS diet.

Table 5.6 Effects of dry distiller's grain with soluble fortified with copper and molybdenum on performance in trial 2

	Diet				
Items	Control	40% DDGS[1]	40% DDGS+[2]	SEM	*P*- value
Initial BW, kg	300	313	312	16	0.82
Final BW, kg	342	351	339	16	0.86
DMI, kg/d	8.07	7.12	7.49	0.47	0.40
ADG, kg	1.08	0.96	0.70	0.16	0.30
Gain: feed	0.132	0.131	0.091	0.02	0.20
DM digestibility, %	73.90[a]	68.34[ab]	65.49[b]	2.23	0.07
OM digestibility, %	74.41	69.57	66.66	2.35	0.11
Mo, ppm	1.63[a]	1.38[a]	9.49[b]	0.40	< 0.01
Cu, ppm	12.89[a]	22.29[a]	111.51[b]	4.84	< 0.01

[1]DDGS- dry distiller's grain with soluble

[2] 40 % DDGS+- supplemented to provide 8 ppm Mo and 90 ppm Cu

[a, b] Means without a common superscript within a row differ ($P \leq 0.10$)

Table 5.7 Effects of distiller's grain with soluble levels on carbon emissions and respiration for phase 1 and 2 during trial 2

	Methane emission									
	Phase 1			Phase 2				*P*- value		
Item	0	40	40 +	0	40	40 +	SEM	TRT	Phase	Phase × TRT
g/d	61.22^{a}	60.68^{a}	40.85^{b}	51.05	46.90	53.07	6.74	0.56	0.28	0.03
g/kg DMI	7.22^{ab}	7.90^{a}	5.34^{b}	9.36	7.56	8.90	0.98	0.67	< 0.01	0.02
g/Mcal DE	3.22^{A}	2.53^{AB}	1.87^{B}	2.67	2.01	2.44	0.33	0.19	0.33	0.03
	Non-methane VOC emission									
g/d	1.50^{A}	0.82^{B}	0.87^{B}	1.36^{A}	0.74^{B}	0.90^{B}	0.15	< 0.01	0.58	0.82
mg/kg DMI	168^{A}	107^{B}	90.6^{B}	238^{A}	107^{B}	135^{B}	-	< 0.01	0.04	0.29
*UCL**	*188*	*120*	*116*	*267*	*121*	*152*	-	-	-	-
*LCL**	*149*	*95.1*	*91.4*	*211*	*95.4*	*120*	-	-	-	-
mg/Mcal DE	77.8^{A}	34.6^{B}	38.8^{B}	71.0^{A}	31.8^{B}	40.6^{B}	6.52	< 0.01	0.62	0.80
	Respiration									
O_2, kg/d	-5.18	-6.18	-6.17	-6.37	-5.95	-4.88	0.73	0.77	0.86	0.28
CO_2, kg/d	5.92^{A}	5.39^{AB}	5.08^{B}	5.18	5.09	4.81	0.20	0.12	< 0.01	0.12
CO_2, g/kg DMI	681	730	649	921	831	797	53.0	0.54	< 0.01	0.06
CO_2, g/ Mcal DE	308^{A}	237^{B}	233^{B}	268	221	221	20.2	0.09	< 0.01	0.11

$^{a, b}$ Means without common superscripts within a row for each phase differ ($P \leq 0.10$)

$^{A, B}$ Means without common superscripts within a row for each phase differ ($P \leq 0.05$)

* Upper and lower confidence limits (exponent of untransformed mean ± 1 SEM) express reliability of the estimated mean emissions. Emissions adjusted for DMI were back-transformed from a natural log transformation to satisfy normal distribution and equal variance

Phase 1

Mean emissions reported were collected from d 12 to 15 (Table 5.7). The TEI 55C analyzer that detects CH_4 and VOC emissions had a malfunction on d 17 to 21. Only CH_4 adjusted for DMI tended to show a treatment × day interaction ($P = 0.08$; data not shown). Non-methane VOC emissions expressed as a mass output and adjusted for DMI both had a highly significant day effect ($P < 0.01$) and a tendency for a treatment difference ($P < 0.10$; data not shown). Carbon dioxide and O_2 consumption also had significant day effects ($P = 0.02$ and $P = 0.04$, respectively). The daily variation suggests longer measurement periods are needed.

No differences were determined in CH_4 emissions between the control and 40% DDGS diets (Table 4.7). However, the addition of Mo and Cu at 8 and 80 ppm, respectively, tended to decrease CH_4 emissions compared to the control and 40% DDGS diets when expressed as a mass output and when adjusted for DMI ($P \leq 0.10$). Methane emissions adjusted for DE were also decreased as a result of feeding the 40% DDGS+ diet compared to the control diet ($P \leq 0.05$). Non-methane VOC emissions were lower in both DDGS diets compared to the control when expressed as mass output, adjusted for DMI, and when adjusted for DE ($P \leq 0.05$). Non-methane VOC adjusted for DMI had to be log transformed to satisfy the assumptions of normality and homoscedasticity, which is why upper and lower confidence intervals are reported rather than reporting the standard error of the mean. Carbon dioxide was decreased in the 40% DDGS+ diet compared to the control when expressed as a mass output and adjusted for DE ($P \leq 0.05$).

Phase 2

The mean emissions reported in phase 2 (Table 5.7) represent d 26 to 29 when cattle were fitted with fecal bags. Fecal bags were placed on the steers on d 24, allowing a 2 d adjustment period prior to days used for analysis. Methane reported as mass output had a significant treatment × day interaction ($P = 0.01$; data not shown) and non-methane VOC adjusted for DMI showed a tendency for a treatment × day interaction ($P = 0.09$; data not shown). Methane adjusted for DMI, VOC mass output, VOC output/DMI, CO_2 mass output, and O_2 consumption all had significant day effects ($P < 0.01$; data not shown).

Methane emissions adjusted for DMI were significantly different in phase 2 compared to phase 1 ($P < 0.01$; Table 5.7). Methane mass output, CH_4 adjusted for DMI, and DE had a significant phase × treatment interaction ($P < 0.05$). All parameters of VOC emissions showed a significant treatment difference ($P < 0.01$) with VOC emissions showing a phase difference when adjusted for DMI ($P = 0.04$). Similar to phase 1, VOC emissions were decreased in the 40% DDGS diets compared to the control ($P \leq 0.05$). All parameters of CO_2 were significantly different in phase 2 compared to phase 1 ($P < 0.01$). However, CO_2 mass output and CO_2 adjusted for DE were decreased in phase 2, whereas CO_2 adjusted for DMI increased in phase 2 compared to phase 1. Carbon dioxide adjusted for DMI also had a tendency for a phase × treatment interaction ($P = 0.06$).

In trial 2, all the N_2O data was recorded as a negative number. This problem has previously occurred during winter months. Perhaps the relative humidity or gases from the heating unit were responsible. Feed, fecal, and urine samples collected in phase 2 during trial 2

were used to calculate an energy balance (Table 5.8). Intake energy and energy losses were similar among treatments. However, the 40% DDGS diets did have a greater NEg compared the control diet ($P < 0.01$).

Table 5.8 Effects of distiller's grain with soluble on energy partition during trial 2 (MJ/d)

	Diet					Contrast	
Item	Control	40% DDGS	40% DDGS+	SEM	*P*-value	Cont vs. DDGS	40 vs. 40 +
Gross energy intake	122.81	143.32	142.95	10.68	0.34	0.15	0.98
Feces	41.52	43.50	50.51	3.40	0.20	0.22	0.18
Urine	2.98	3.29	3.38	0.32	0.68	0.40	0.87
Methane	2.72	2.75	2.97	0.37	0.88	0.78	0.69
Total loss	47.23	49.54	56.86	3.72	0.22	0.22	0.20
Digestible energy	81.28	99.82	92.44	8.22	0.32	0.17	0.54
Metabolizable energy	75.58	93.78	86.10	7.93	0.31	0.17	0.51
Heat energy[1]	63.97	58.46	46.65	7.22	0.27	0.23	0.28
Net energy	11.61[a]	35.32[b]	39.45[b]	5.97	0.02	< 0.01	0.64

[1] HE = 16.26 KJ × O_2 (L/d) + 5.02 KJ × CO_2 (L/d) – 2.17 KJ × CH_4 (L/d) – 5.99 KJ × N (g/d); Brouwer (1965)

[a, b] Means within a row without common superscripts differ

DISCUSSION

Methane emissions were similar in phase 1 and 2 when cattle were fitted with fecal bags to separate urine and feces. This would suggest that CH_4 production is enteric and emitted through eructation and flatulation. Compared to the control and 60% DDGS diets, steers fed the 40% DDGS diet tended to perform better based on ADG and DM and OM digestibilities ($P < 0.10$) while emitting the least amount of greenhouse gases ($P = 0.08$). Cattle fed the 40% DDGS diet emitted the least amount of GHG and numerically lowest CH_4 production in both phase 1 and 2 during trial 1. One explanation for a decrease in GHG and CH_4 in the 40% DDGS diet may be that CH_4 acts as an energy sink, collecting H from rumen microorganisms (Fahey and Berger, 1988). The DDGS diets were greater in dietary N and S, which may provide competing H sink sources in the ruminal gas cap; resulting in less H to yield formate (**HCOOH**), which actively converts to CH_4 by methanogens (Fahey and Berger, 1988). Hydrogen sulfides that are formed in the ruminal gas cap are likely absorbed by inhalation into the lungs (NRC, 2005; Crawford, 2007) where H_2S molecules enter the bloodstream and are eventually excreted as sulfates in the urine. Therefore, NH_3 and H_2S gases may still be produced at high enough concentrations in the rumen to compete with HCOOH for protons and limit formation of CH_4 gas, despite enteric sources contributing very little NH_3 and H_2S emissions from eructation. Other studies support this argument, as Loneragan et al. (1998) reported H_2S in the ruminal gas cap as high as 19 mg/L or 13,500 ppm in cattle fed high S diets. Additionally, volatile fatty acid (**VFA**) production from ruminal microorganisms are likely to influence the amount of CH_4

produced. An in-vitro study conducted by Behlke et al. (2007) reported a linear reduction of CH_4 produced per g of digested DM when replacing brome hay with various levels of DDGS at: 0, 25, 50, 75, and 100%. Decreasing the ratio of forage:concentrate also decreases the ratio of acetate:propionate and CH_4 production (Annison et al., 1970; Van Soest, 1982; Fahey and Berger, 1988; Behlke et al., 2007).

The 40% DDGS+ diet in trial 2 showed a tendency to reduce CH_4 and CO_2 emissions. This may be the result of the sensitivity of methanogenic bacteria to dietary conditions. Increased passage rate, increased fermentation, and decreased pH often improve animal performance as the C and H lost in CH_4 production is decreased and retained in propionate, increasing ME in the diet (Fahey and Berger, 1988). Within trial 2 of the current study, the 40% DDGS diets had a numerically greater ME and showed a significant increase in NE compared to the control (P = 0.02). In trial 1 and 2, fecal loss accounted for approximately 30-35% of the gross IE. A study conducted by Tyrrell et al. (1988) reported loss from FE to be around 35% in diets fed to lactating cows. Kurihara et al. (1999) reported a FE loss near 30% when feeding a high grain diet to Brahman heifers. Other reports suggest that of the total IE, approximately 45% is lost as heat, 40% lost as feces, 10% lost as urine and gas, leaving only 5% of the energy retained for growth (Ferrell, 1988). In both trials, energy lost as heat accounted for 33 to 52% of the total IE. Energy loss from CH_4 gas is low within the current study compared to others. However, the previous studies fed diets containing a greater amount of forage compared to the diets fed in this study. Consequently, NE in the current study is greater in comparison to other reports. Estimation of the net energy value of DDGS was 0.93 Mcal/kg DMI when subtracting the estimated tabular values for CS and SBM (Figure 5.1). The improved performance when feeding the 40% DDGS diet

compared to the control and 60% DDGS diets infers a large positive associative effect. This may be reflected in improved ruminal fermentation, but this was not measured in the current study. Furthermore, Figure 5.1 would suggest that feeding DDGS in moderate levels, between 25 to 40% shows the greatest efficiency in converting IE to NEg.

All livestock species are reported to emit 58 million tons/yr or 73% of the total annual emitted CH_4 each year (US Environmental Protection Agency, 1994). The U.S. Environmental Protection Agency (2000) reported that the average passenger car emits 95 g/d of hydrocarbons and over 14 kg/d of CO_2. Additionally, a small truck emits 133 g/d of hydrocarbons and just under 20 kg/d of CO_2. In comparison, steers from our study that weighed around 300 kg and consumed about 7 kg DMI/d concentrate diet, emitted approximately 30 to 60 g/d of hydrocarbons (CH_4 and VOC) and 3.5 to 6.0 kg/d of CO_2 per steer. Based on these results, a passenger car emits 2- fold the hydrocarbons and a small truck emits nearly 3- fold the hydrocarbons compared to the emissions generated from steers within the current study. Carbon dioxide emitted from a passenger car and small truck is nearly 3 and 4- fold greater, respectively, than the CO_2 emitted by the steers within our study.

Based on our results, steers fed concentrate diets consisting of either corn or DDGS produce less CH_4 compared to estimates from cattle fed forage-based diet and therefore have a greater NE. This energy efficiency results in less overall GHG as well. However, globally the majority of cattle are fed in a grazing system. In order to mitigate the GHG footprint from livestock globally, further research should consider strategies to reduce GHG in livestock reliant on forage-based feeds without sacrificing the health and performance of the animal. Within more

intensive feeding systems or feedlots, further research could provide stronger evidence that feeding moderate levels of DDGS (20 to 40%) generates lower GHG compared to other concentrate diets. Furthermore, more research should be conducted on the effects of supplementing Cu and Mo may have on ruminant health and reduction of CH_4 emissions.

CHAPTER 6: CONCLUSIONS

The current study provides evidence that manure is the main source of H_2S and N-emissions. The H_2S gas produced in the rumen was likely absorbed in the lungs or lower GI tract as protonated sulfides are unable to be absorbed through the rumen epithelium (NRC, 2005; Crawford, 2007). Hydrogen sulfide and NH_3 emissions from the manure (urine and feces) did not linearly increase as dietary inclusion of DDGS increased from 0 to 60%. Hydrogen sulfide emissions tended to demonstrate a quadratic effect, as H_2S emissions increased with the 40% DDGS diet and decreased at 60%. No dietary differences were determined for NH_3 emissions or greenhouse gases (**GHG;** CH_4, CO_2, N_2O). Cattle fed the 60% DDGS diet tended to have lower ADG and DM digestibility. This suggests that feeding DDGS at 60% inclusion or greater may negatively affect ruminal fermentation, and also explain the decreased H_2S emissions in the 60% DDGS diet compared to the 40% DDGS diet. Others have reported diminishing performance when DGS inclusion levels exceed 45% of the dietary DM (Gordon et al., 2002; Gunn et al., 2009). Further research looking at mitigation strategies for these gases should consider waste treatment strategies rather than dietary treatments to the animal.

Including Mo and Cu in the diet tended to decrease manure H_2S emissions. The decrease of H_2S emissions could be an effect of insoluble compounds (MoS_4Cu) forming in the manure (Hamsell et al., 2010). Another possible explanation is Mo and Cu have inhibitory effects on sulfate-reducing bacteria (SRB), decreasing H_2S emissions from the manure. Molybdate has been reported to decrease H_2S production in ruminal gas caps by inhibition of SRB (Oremland

and Capone, 1988; Loneragan et al., 1998; Kung, 2008). Therefore, it is reasonable to consider this inhibitory effect may occur in the manure as well. The tendency for Mo and Cu to decrease H_2S emissions should be further researched with more animals per treatment. Additionally, measurements of tetrathiomolybdates in the blood and feces could help determine if Mo and Cu are forming insoluble compounds. Studies could be performed as well to test the potential inhibitory effects of Mo and Cu on SRB in the rumen and feces.

The use of Mo and Cu with a 40% DDGS diet also showed tendencies to decrease CH_4 emissions. However, further research should consider using a treatment with high forage diets that produce greater levels of CH_4 gas than the high concentrate diets used in the current study. Additionally, decreasing energy loss from CH_4 production could improve animal performance and offer a financial incentive to producers. Methane emissions may have decreased as a result of rumen microbial sensitivity to an increase in dietary Mo and Cu (Jones et al., 1982). It is unclear how Mo and Cu may be decreasing CH_4 gas. Future studies should consider looking at the impact of Mo and Cu on methanogens and ruminal CH_4 gas production.

The rate of excreted S and N linearly increased with greater concentrations of DDGS in cattle diets. In both trials, the 40% DDGS diets increased S and N digestibility compared to the control diet. Sulfur and N retention were also increased in the 40% DDGS diets in trial 2. However, S and N retention were similar in trial 1. Increasing levels of DDGS fed to cattle provides greater concentrations of dietary methionine and cysteine. Some of the increased excretion of S may be explained by the greater levels of S-containning amino acids the the DDGS diets. Metabolism of methionine and cysteine results in elevated S excretion as sulfates in

urine (Fron et al.,1990). Gross intake energy and total energy loss did not differ from increasing concentrations of DDGS in cattle diets. Urine energy decreased with the inclusion of DDGS in trial 1 but did not differ in trial 2. Conversely, net energy gain was greater in the 40% DDGS diets compared to the control diet in trial 2 but was similar in trial 1. Overall, feeding DDGS at moderate levels does increase H_2S and NH_3 emissions compared to traditional corn-based diets. The use of Mo and Cu may decrease manure H_2S emissions and ruminal CH_4 gas production.

APPENDICES

APPENDIX A

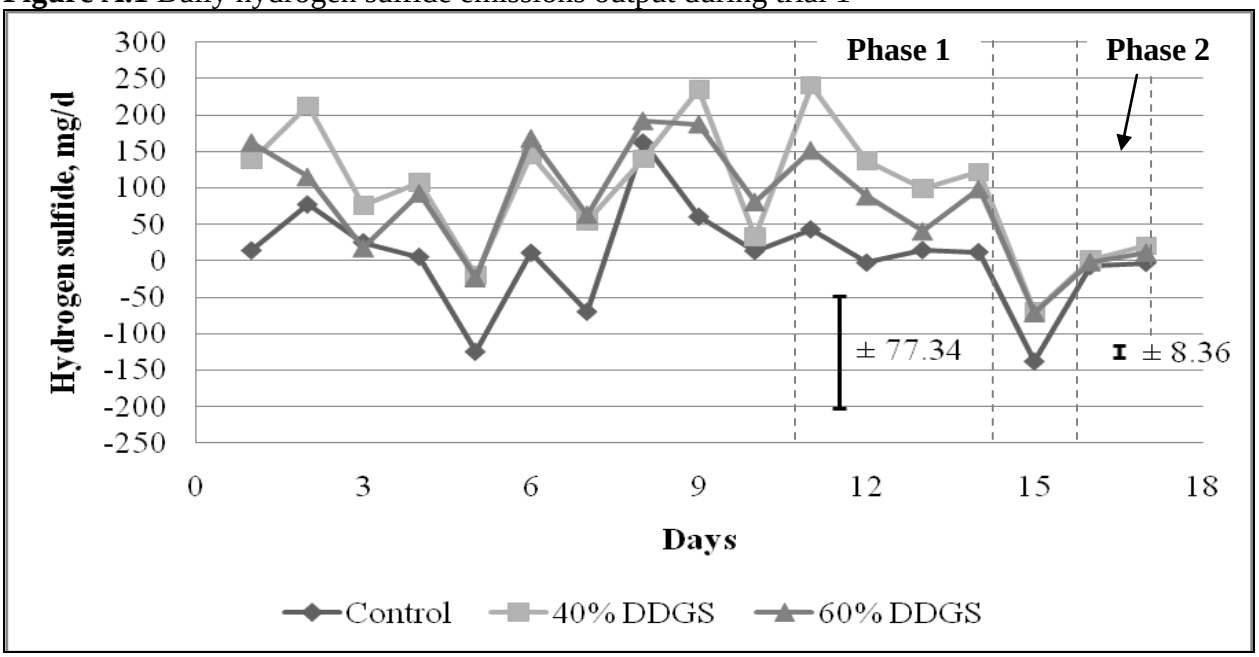
Trial 1

Table A.1 Comparison of unfiltered raw data to filtered data during trial 1

	Unfiltered raw data*				
Variable	N	Mean	Standard Deviation	Minimum	Maximum
Temperature, ºF	18547	58.863	3.944	49.108	68.569
Humidity, %	18547	49.663	11.234	24.896	104.000
Air flow, cfm	18547	500.968	188.588	0.000	654.083
H_2S, ppm	18547	0.005	0.008	0.000	0.135
SO_2, ppm	18547	0.001	0.001	0.000	0.018
	Filtered raw data*				
Variable	N	Mean	Standard Deviation	Minimum	Maximum
Temperature, ºF	18547	58.863	3.944	49.108	68.569
Humidity, %	18547	49.663	11.234	24.896	104.000
Air flow, cfm	17117	542.821	125.766	106.843	654.083
H_2S, ppm	18541	0.005	0.007	0.000	0.098
SO_2, ppm	18547	0.001	0.001	0.000	0.018

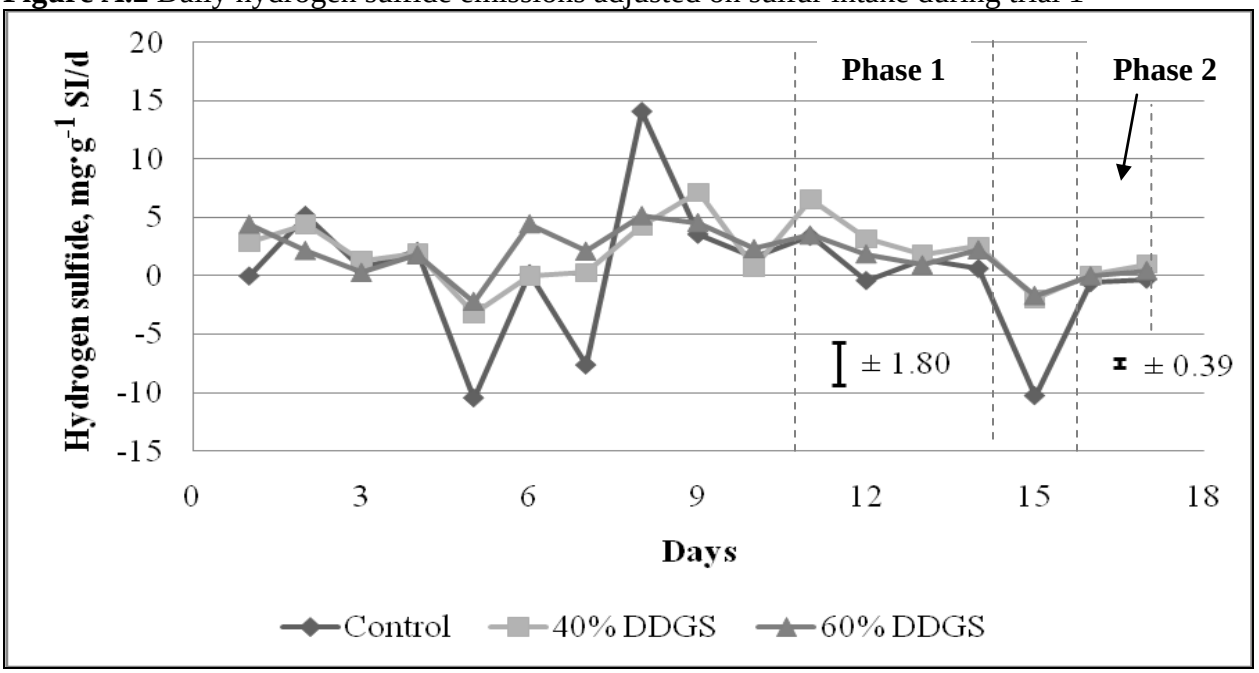
* Raw data represents all of the days cattle were housed within environmentally-controlled rooms

Figure A.1 Daily hydrogen sulfide emissions output during trial 1



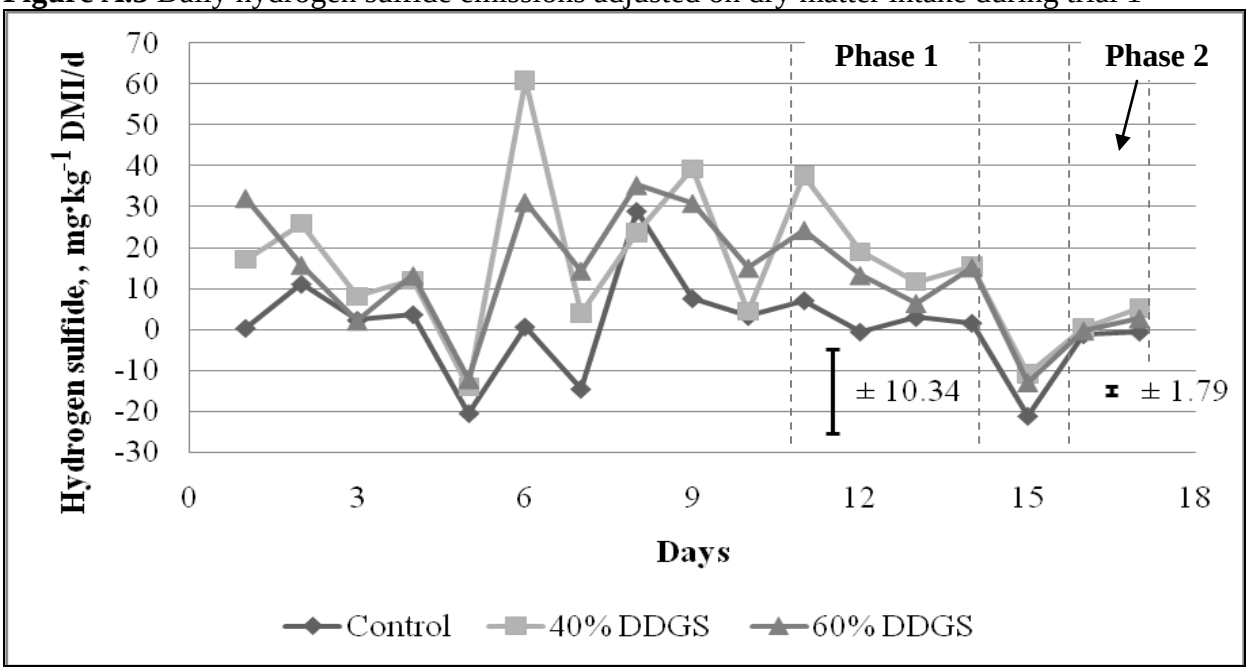
* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure A.2 Daily hydrogen sulfide emissions adjusted on sulfur intake during trial 1



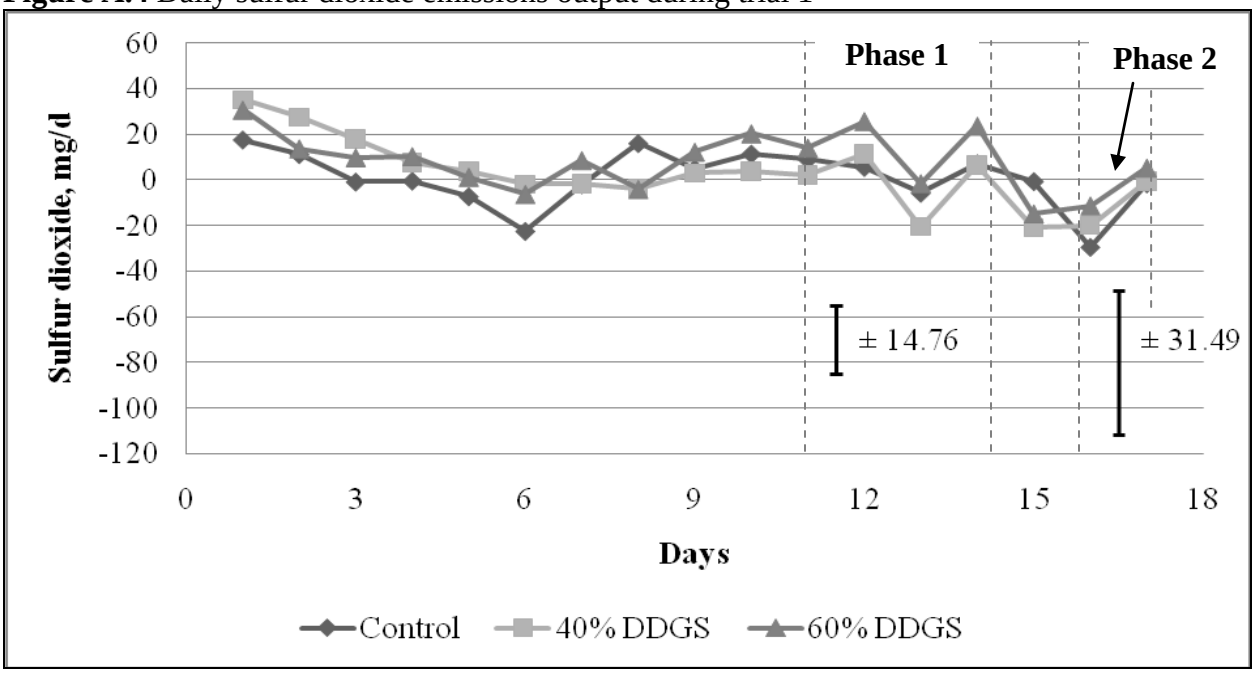
* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure A.3 Daily hydrogen sulfide emissions adjusted on dry matter intake during trial 1



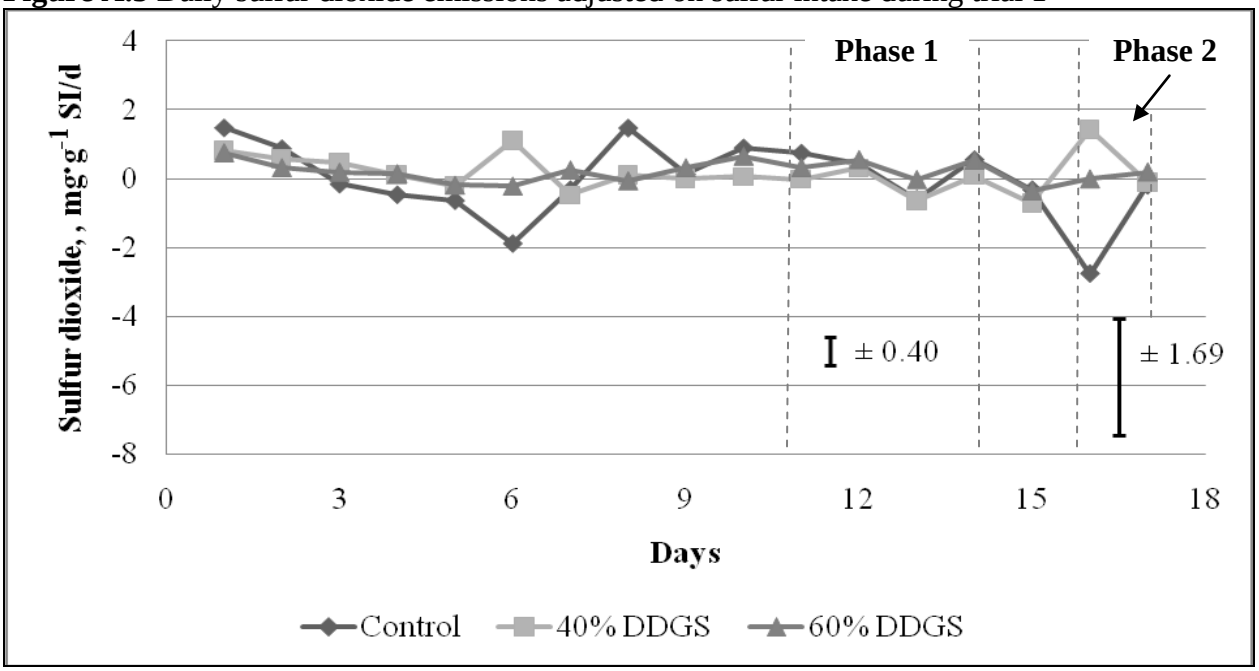
* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure A.4 Daily sulfur dioxide emissions output during trial 1



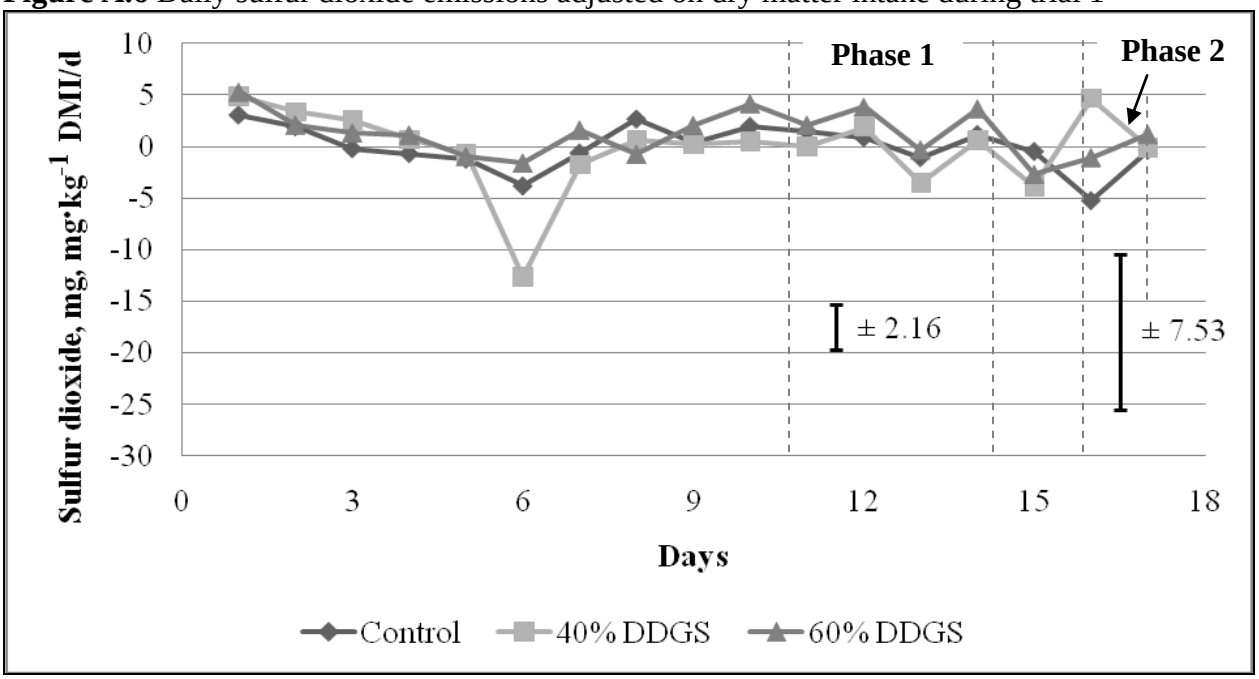
* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure A.5 Daily sulfur dioxide emissions adjusted on sulfur intake during trial 1



* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure A.6 Daily sulfur dioxide emissions adjusted on dry matter intake during trial 1



* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Table A.2 Probability of day effects for sulfur-containing emissions during trial 1

	Phase 1			Phase 2		
Variable	TRT	Day	TRT × Day	TRT	Day	TRT × Day
H_2S, mg/d	0.36	< 0.01	0.52	0.26	0.02	0.25
H_2S, mg/g SI	0.51	< 0.01	0.68	0.10	0.07	0.59
H_2S, mg/kg DMI	0.41	0.01	0.69	0.31	0.02	0.40
SO_2, mg/d	0.59	< 0.01	0.50	0.81	0.88	0.73
SO_2, mg/g SI	0.61	< 0.01	0.39	0.48	0.73	0.40
SO_2, mg/kg DMI	0.66	< 0.01	0.44	0.92	0.36	0.98

Figure A.7 Mean hydrogen sulfide emissions output during trial 1

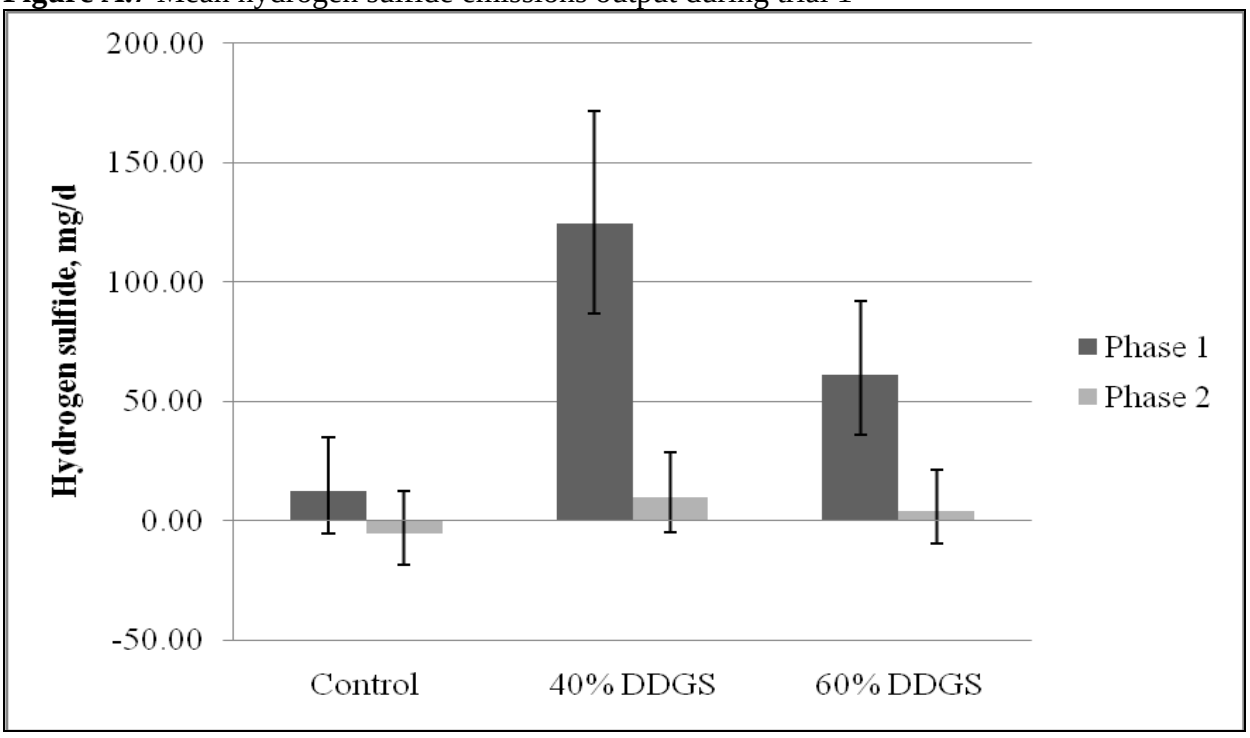


Figure A.8 Mean hydrogen sulfide emissions adjusted on sulfur intake during trial 1

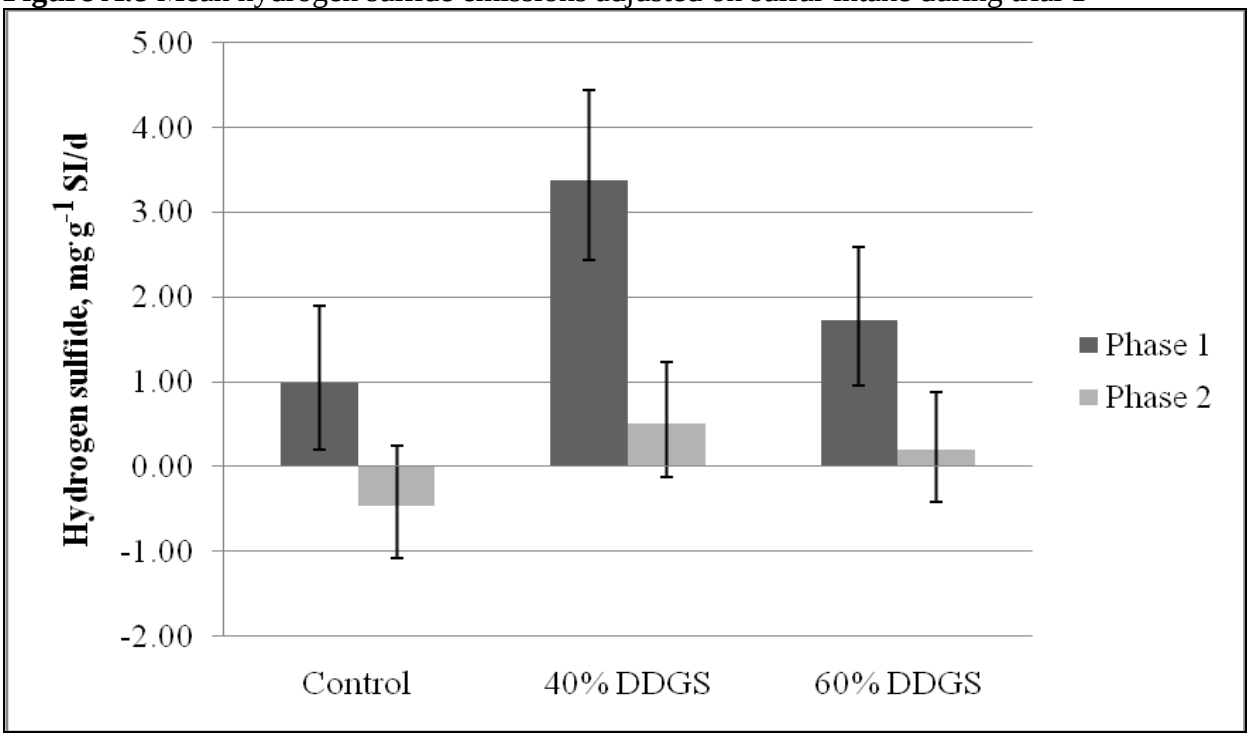


Figure A.9 Mean hydrogen sulfide emissions adjusted on dry matter intake during trial 1

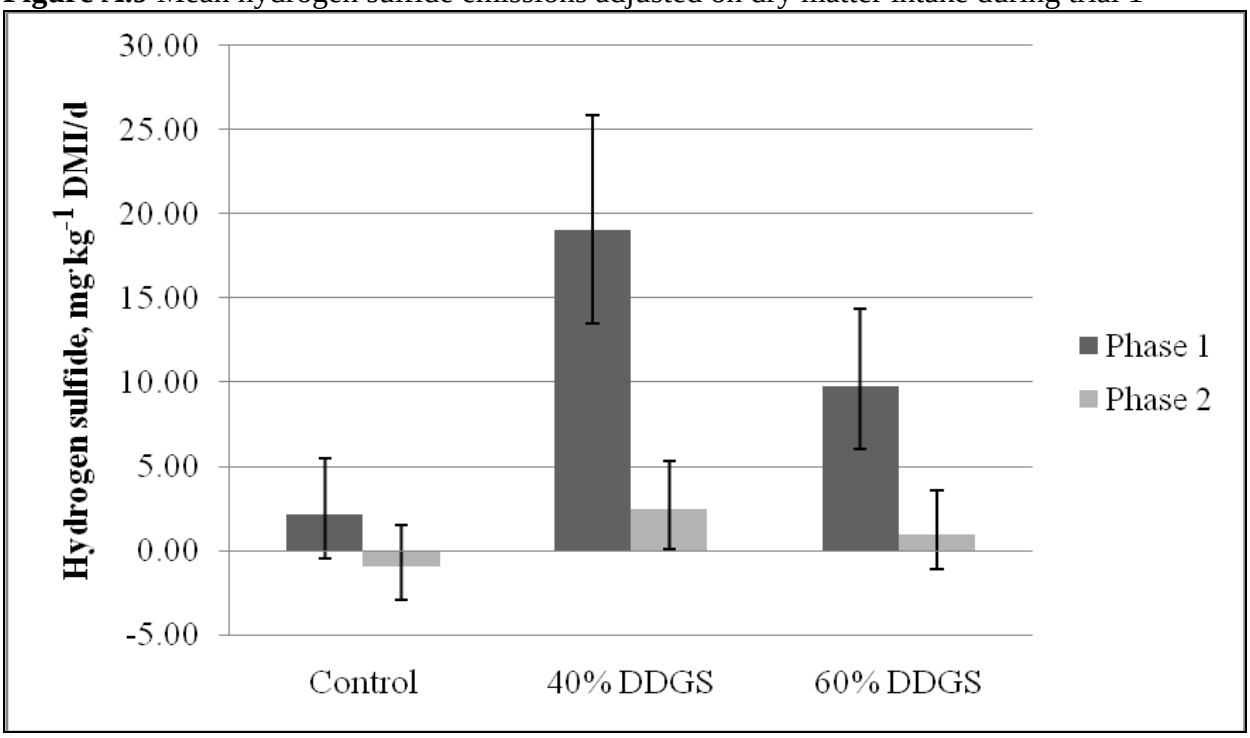


Figure A.10 Mean sulfur dioxide emissions output during trial 1

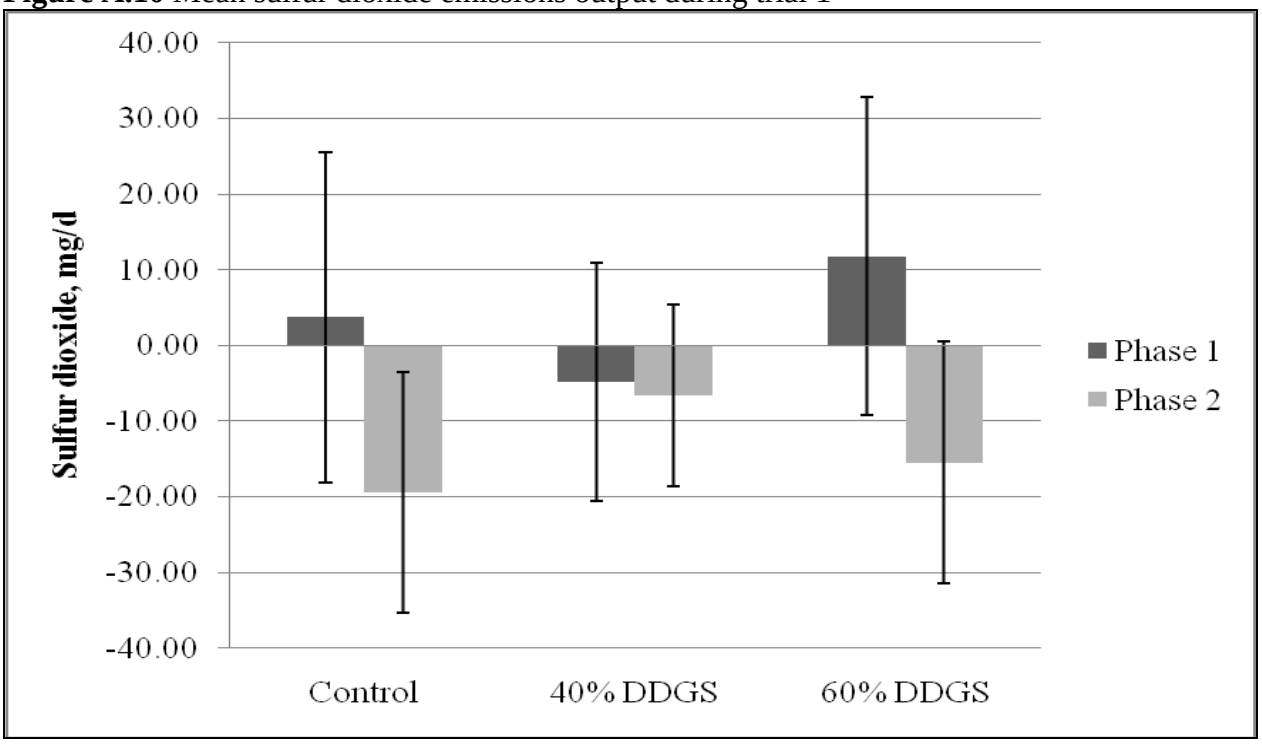


Figure A.11 Mean sulfur dioxide emissions adjusted on sulfur intake during trial 1

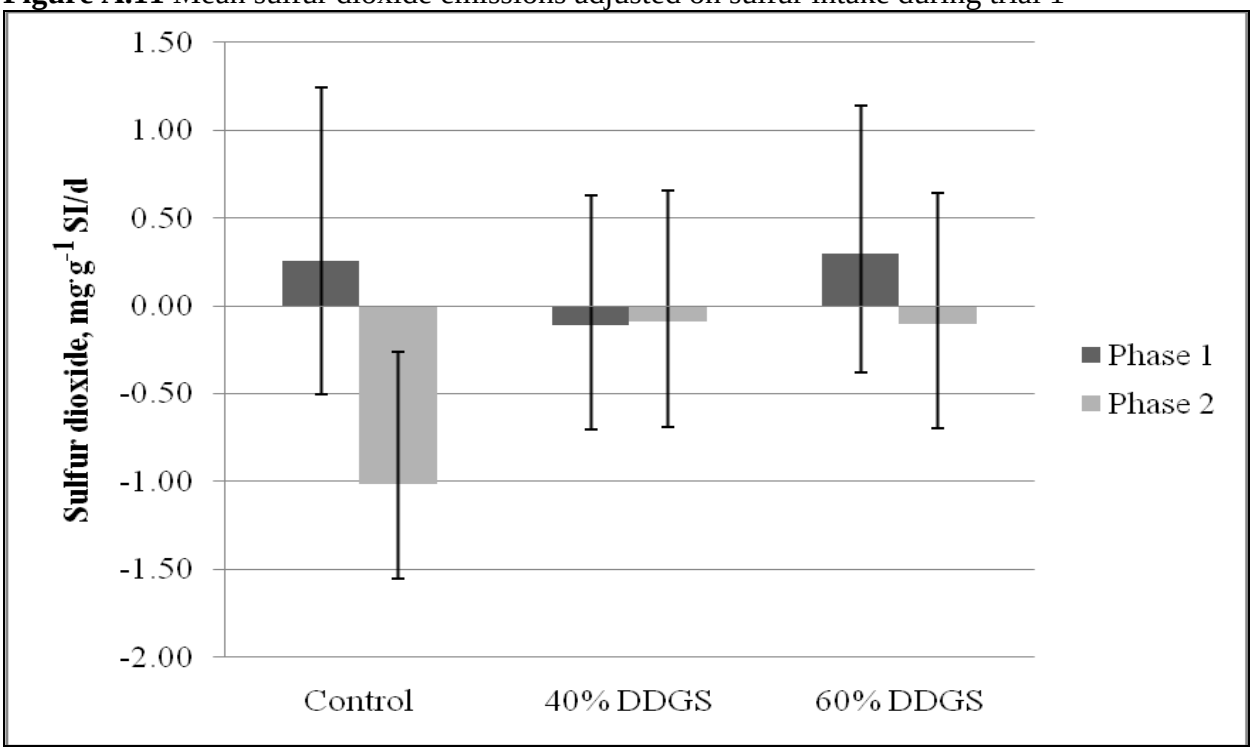
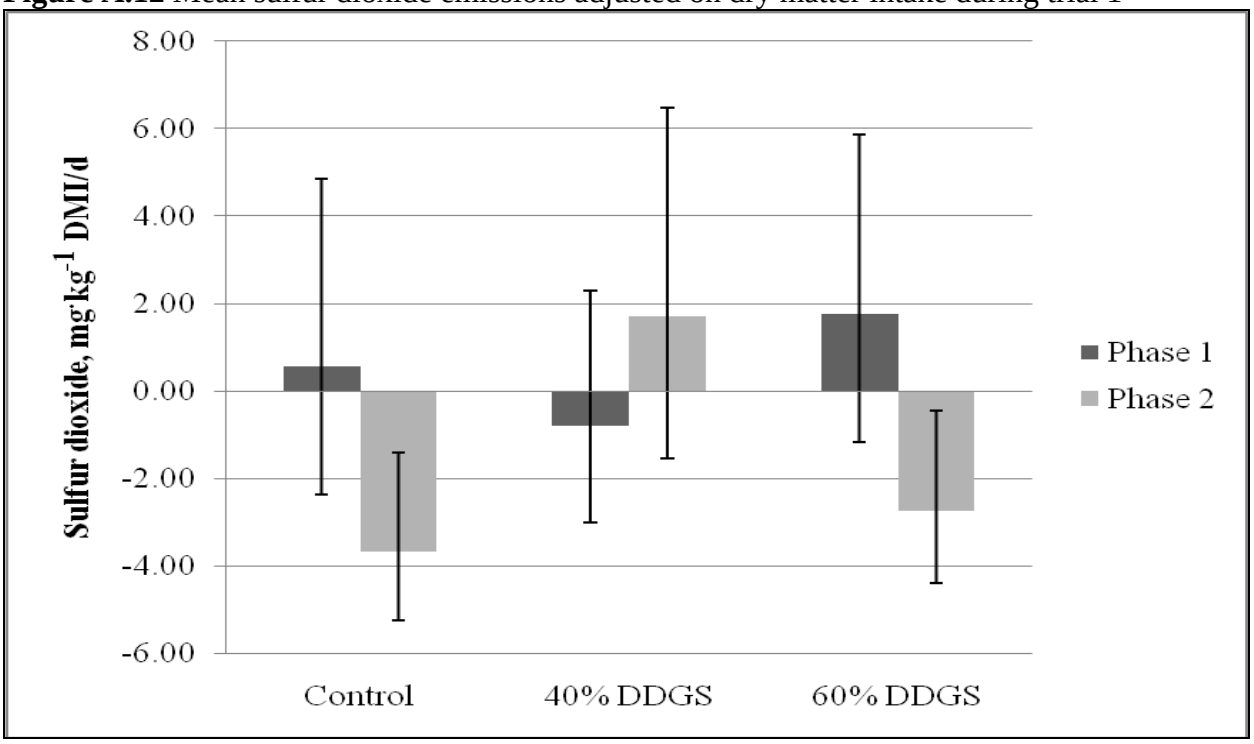


Figure A.12 Mean sulfur dioxide emissions adjusted on dry matter intake during trial 1



Trial 2

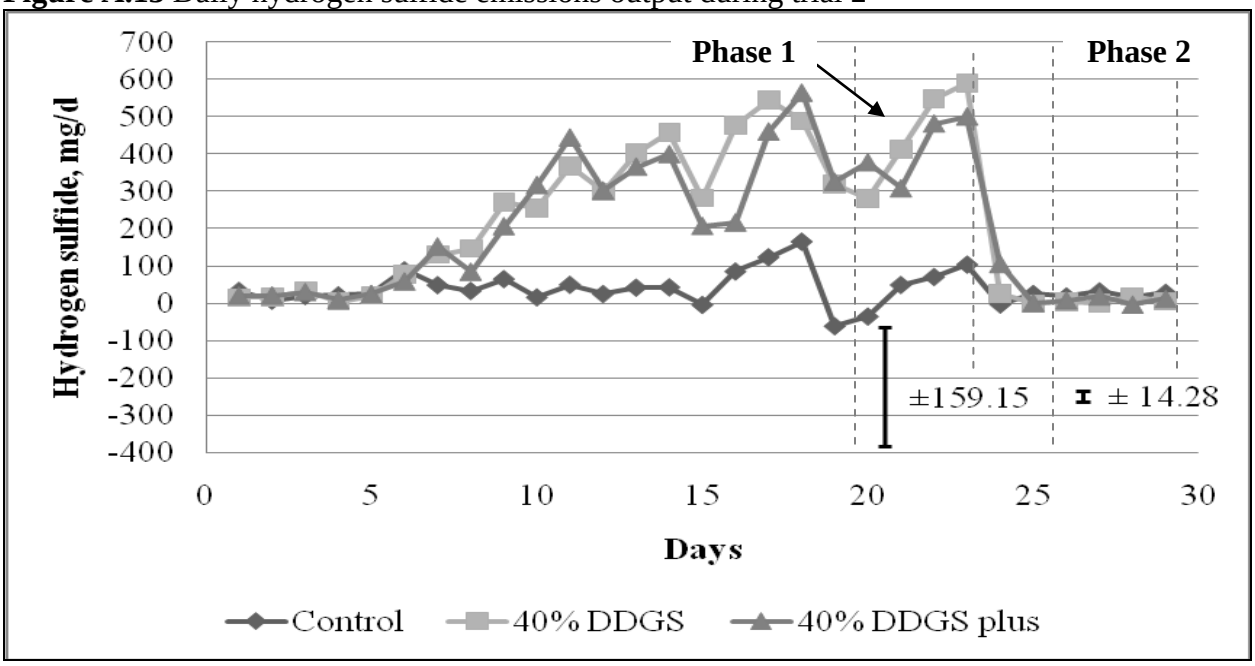
Table A.3 Comparison of unfiltered raw data to filtered data during trial 2

	Unfiltered raw data*				
Variable	N	Mean	Standard Deviation	Minimum	Maximum
Temperature, ºF	28870	60.367	1.813	48.688	65.353
Humidity, %	28870	36.041	11.308	16.109	104.000
Air flow, cfm	28870	545.111	179.405	0.000	659.829
H_2S, ppm	28870	0.010	0.014	-0.003	0.405
SO_2, ppm	28869	0.003	0.001	-0.003	0.009

	Filtered raw data*				
Variable	N	Mean	Standard Deviation	Minimum	Maximum
Temperature, ºF	28870	60.367	1.813	48.688	65.353
Humidity, %	28870	36.041	11.308	16.109	104.000
Air flow, cfm	26609	591.430	86.750	97.005	659.829
H_2S, ppm	28791	0.010	0.012	-0.003	0.100
SO_2, ppm	28869	0.003	0.001	-0.003	0.009

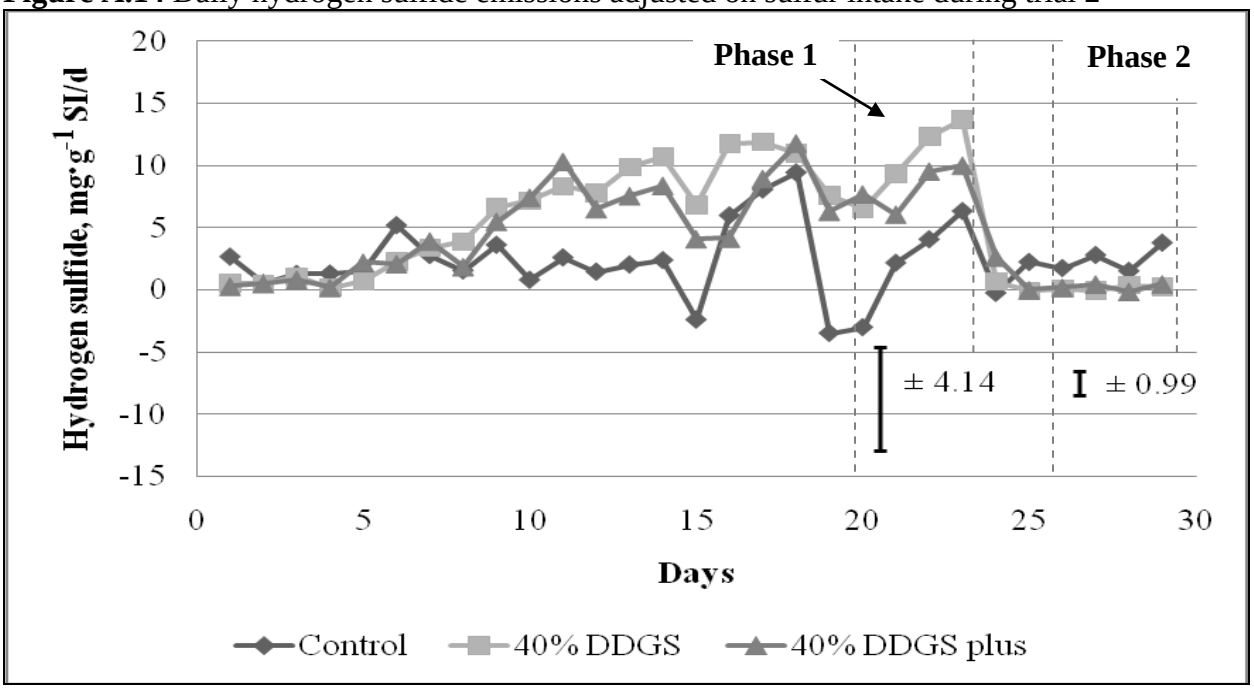
* Raw data represents all of the days cattle were housed within environmentally-controlled rooms

Figure A.13 Daily hydrogen sulfide emissions output during trial 2



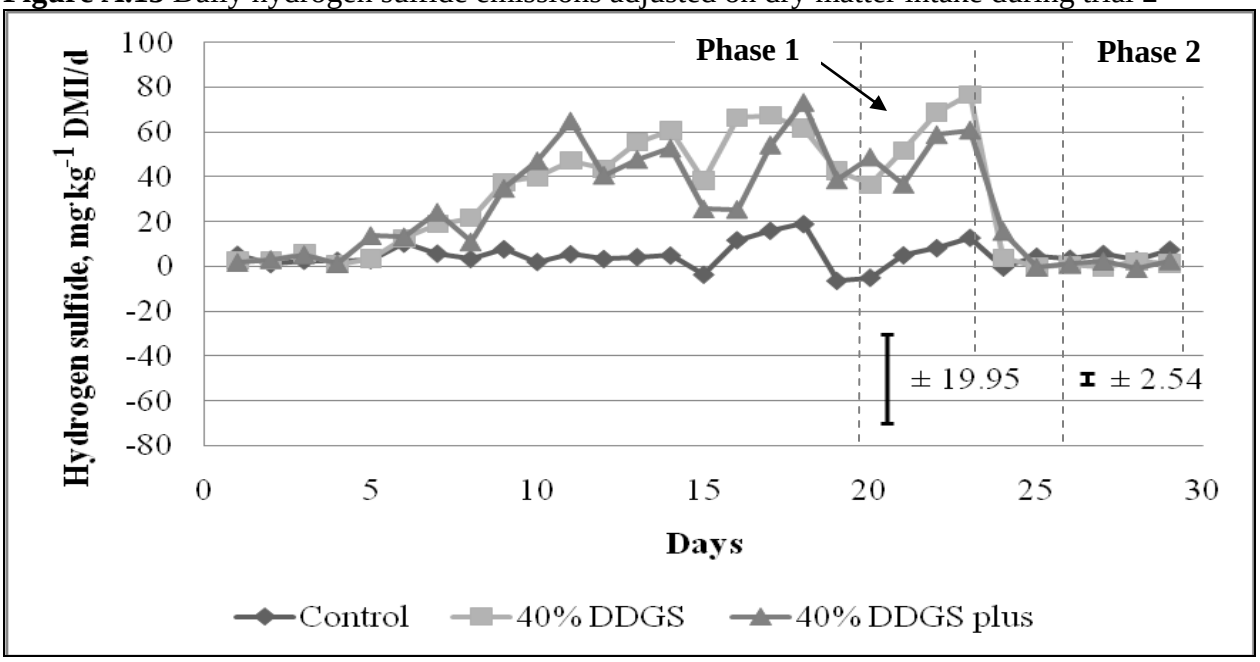
* Days 20-23 represent steady state mean emissions during phase 1. Days 26-29 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure A.14 Daily hydrogen sulfide emissions adjusted on sulfur intake during trial 2



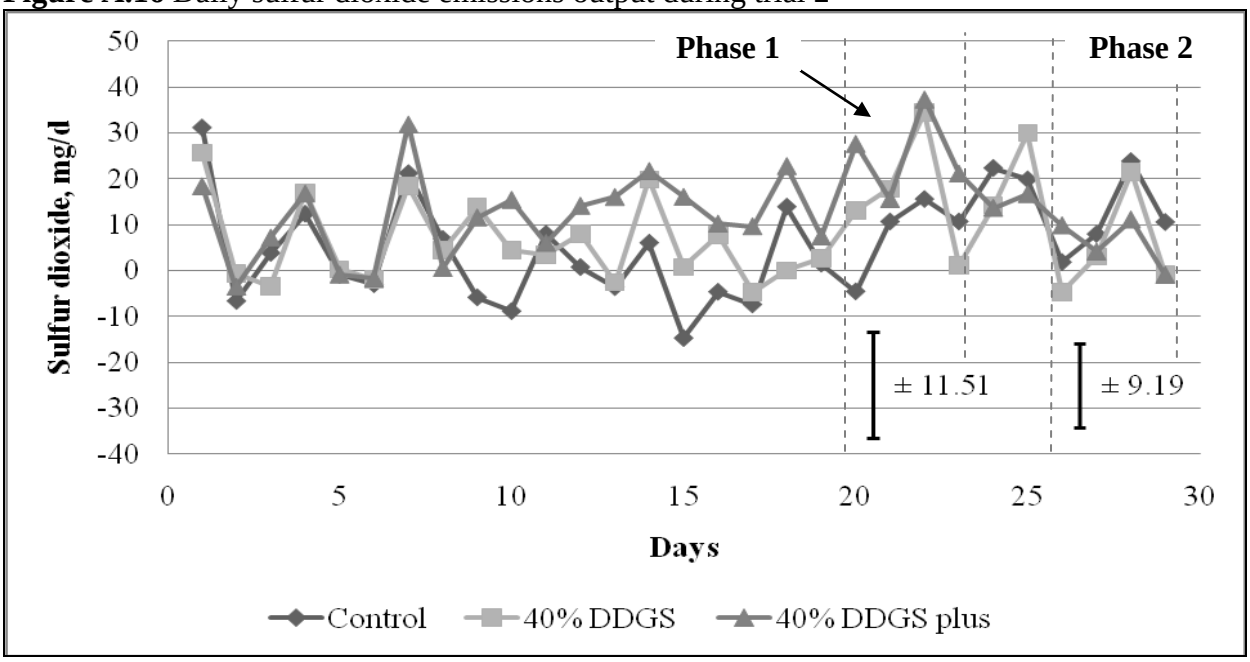
* Days 20-23 represent steady state mean emissions during phase 1. Days 26-29 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure A.15 Daily hydrogen sulfide emissions adjusted on dry matter intake during trial 2



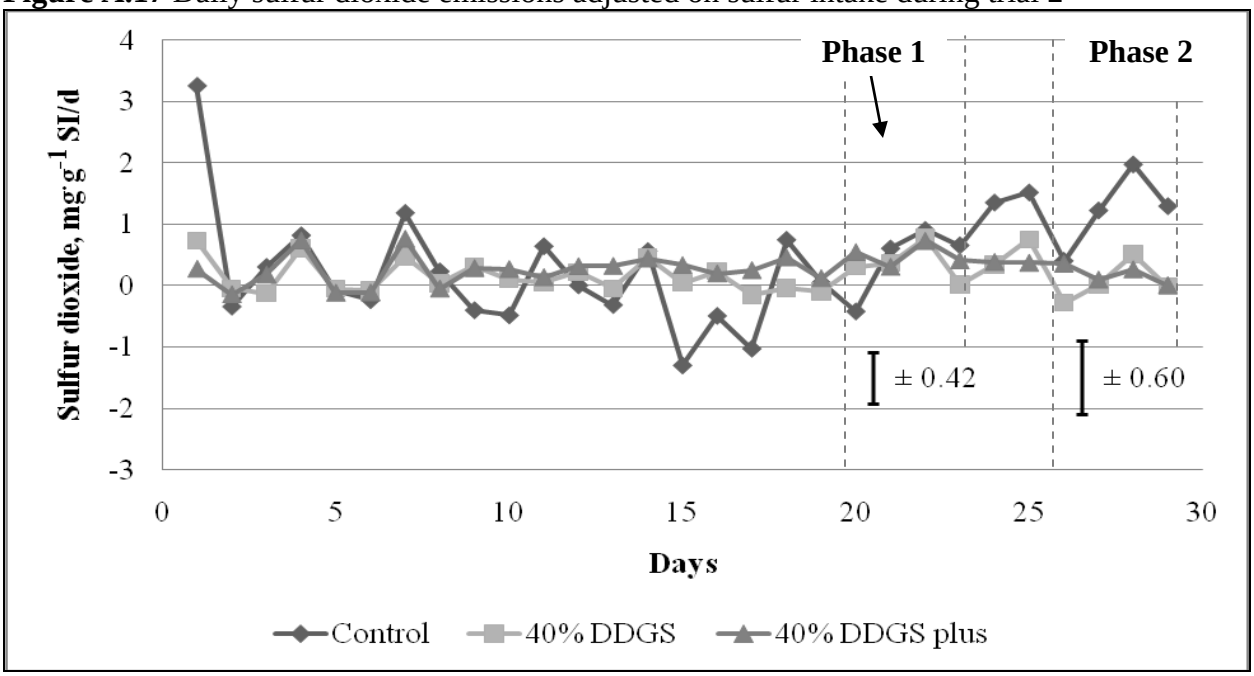
* Days 20-23 represent steady state mean emissions during phase 1. Days 26-29 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure A.16 Daily sulfur dioxide emissions output during trial 2



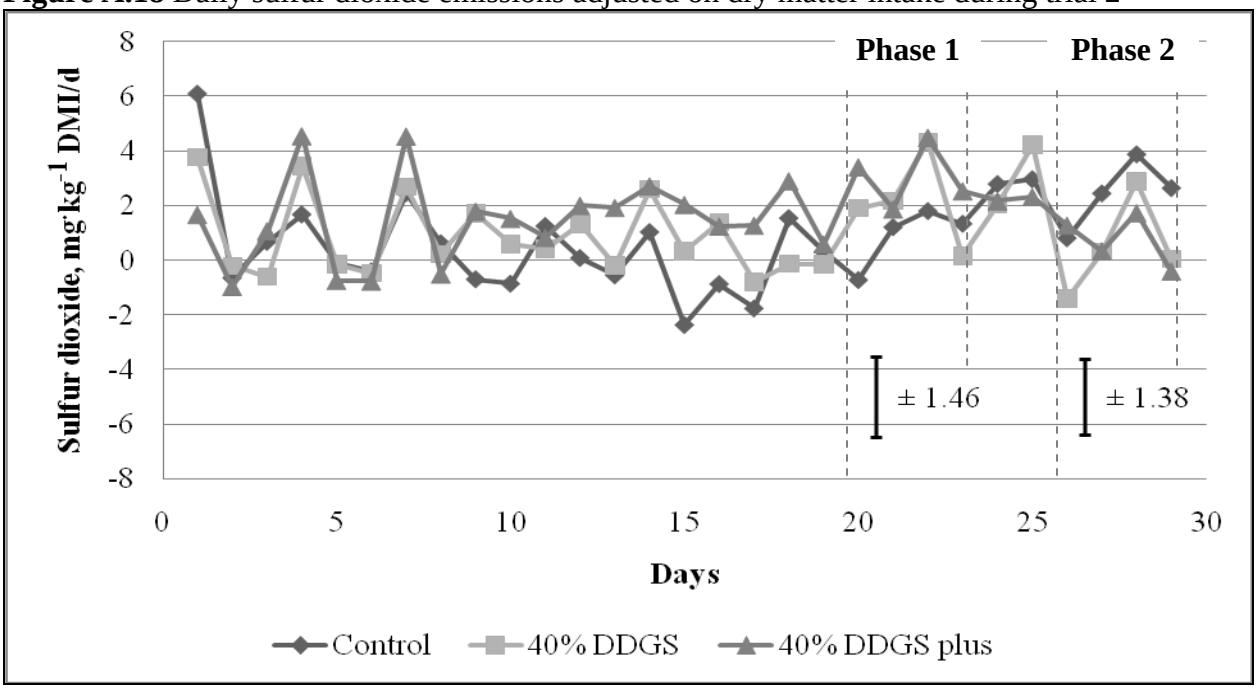
* Days 20-23 represent steady state mean emissions during phase 1. Days 26-29 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure A.17 Daily sulfur dioxide emissions adjusted on sulfur intake during trial 2



* Days 20-23 represent steady state mean emissions during phase 1. Days 26-29 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure A.18 Daily sulfur dioxide emissions adjusted on dry matter intake during trial 2



* Days 20-23 represent steady state mean emissions during phase 1. Days 26-29 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Table A.4 Probability of day effects for sulfur-containing emissions during trial 2

	Phase 1			Phase 2		
Variable	TRT	Day	TRT × Day	TRT	Day	TRT × Day
H_2S, mg/d	0.99	< 0.01	0.38	0.47	0.21	0.64
H_2S, mg/g SI	0.99	< 0.01	0.95	0.73	0.78	0.63
H_2S, mg/kg DMI	0.92	< 0.01	0.61	0.56	0.53	0.87
SO_2, mg/d	0.55	< 0.01	0.35	0.14	< 0.01	0.99
SO_2, mg/g SI	0.39	0.01	0.71	0.53	< 0.01	0.90
SO_2, mg/kg DMI	0.55	< 0.01	0.42	0.07	< 0.01	0.89

Figure A.19 Mean hydrogen sulfide emissions output during trial 2

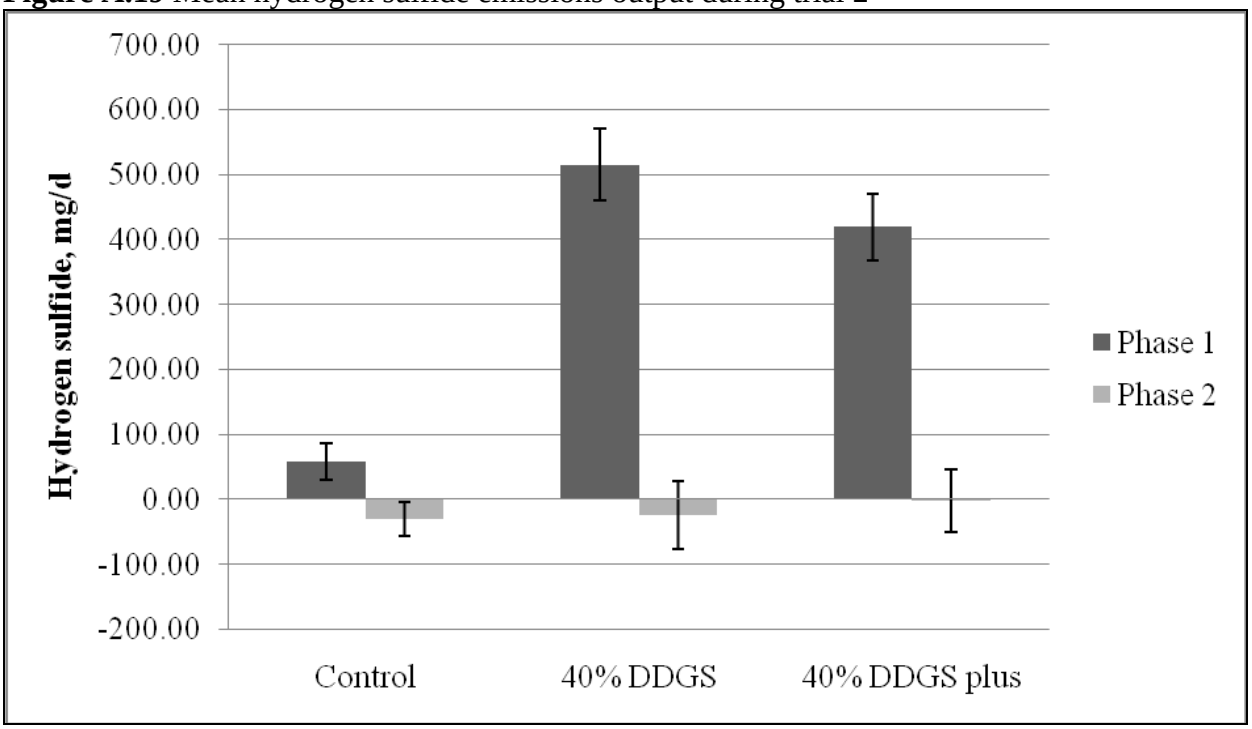


Figure A.20 Mean hydrogen sulfide emissions adjusted on sulfur intake during trial 2

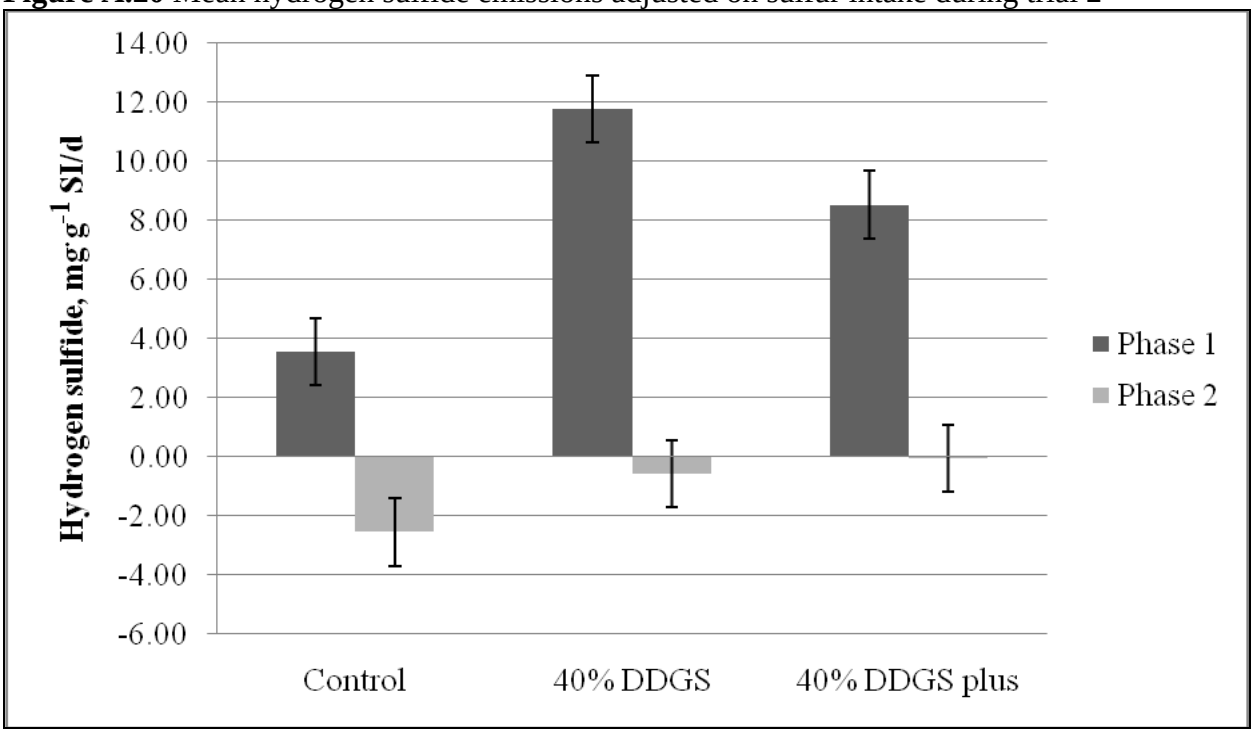


Figure A.21 Mean hydrogen sulfide emissions adjusted on dry matter intake during trial 2

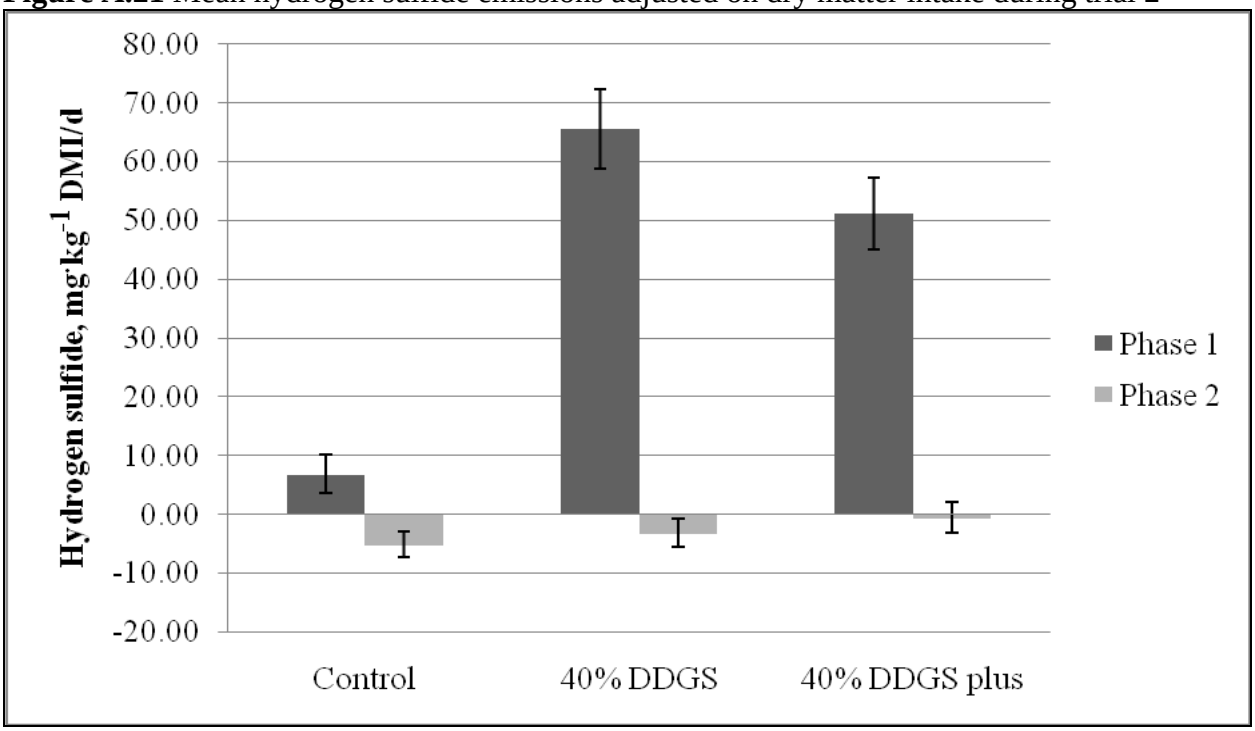


Figure A.22 Mean sulfur dioxide emissions output during trial 2

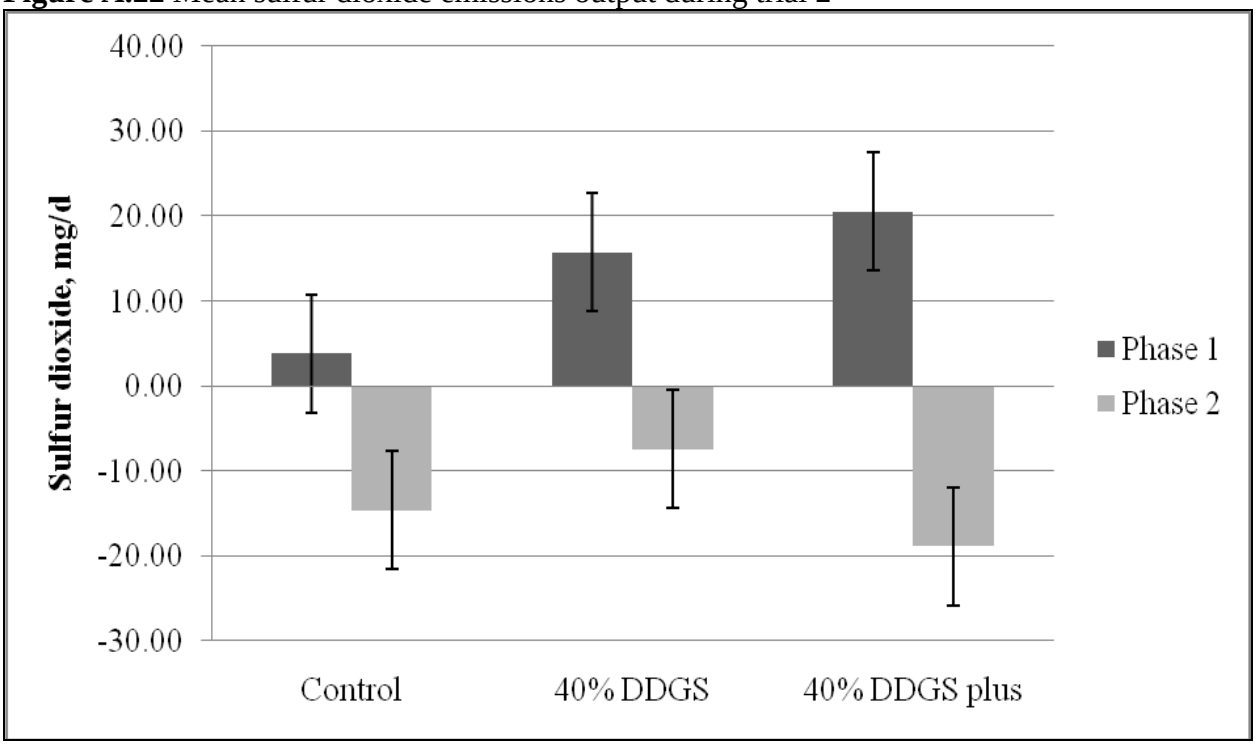


Figure A.23 Mean sulfur dioxide emissions adjusted on sulfur intake during trial 2

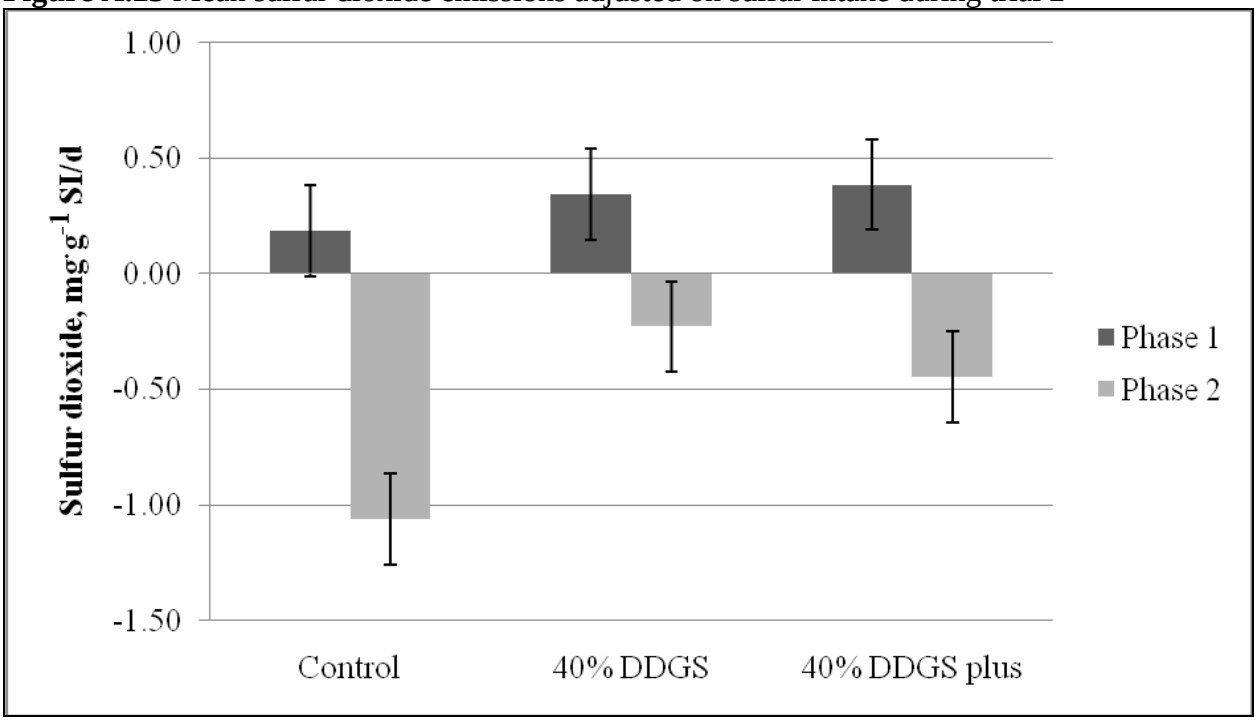
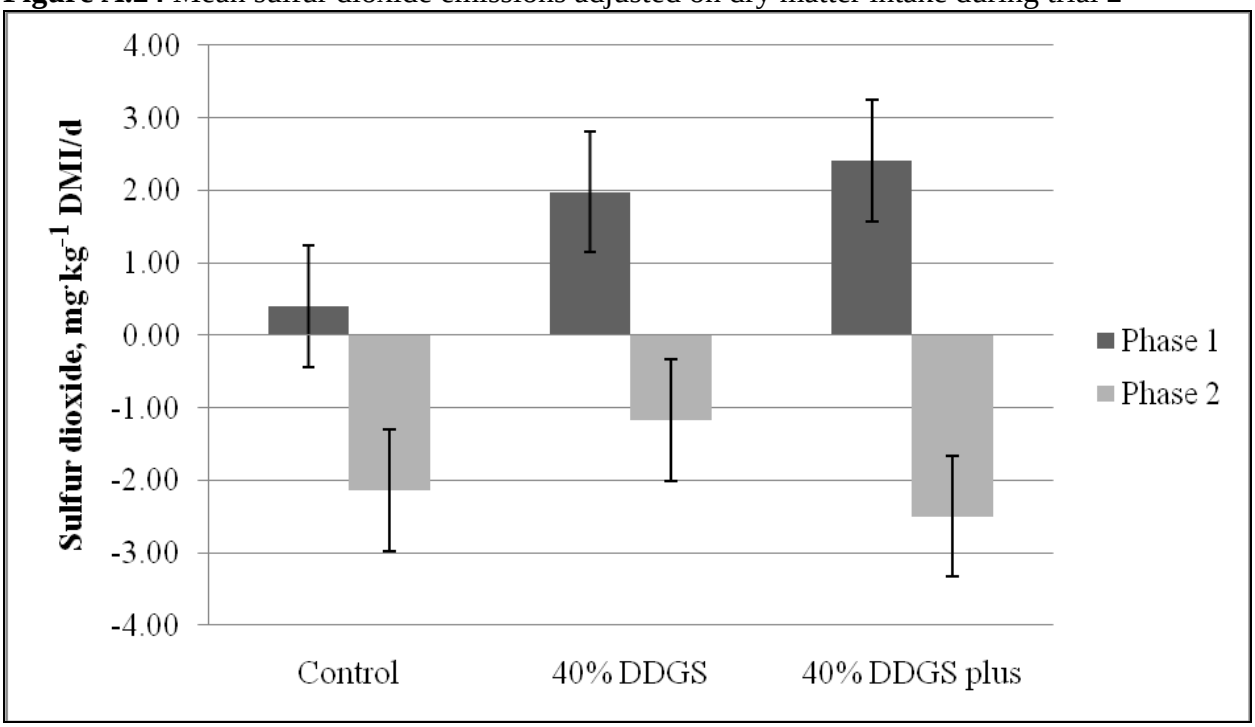


Figure A.24 Mean sulfur dioxide emissions adjusted on dry matter intake during trial 2



APPENDIX B

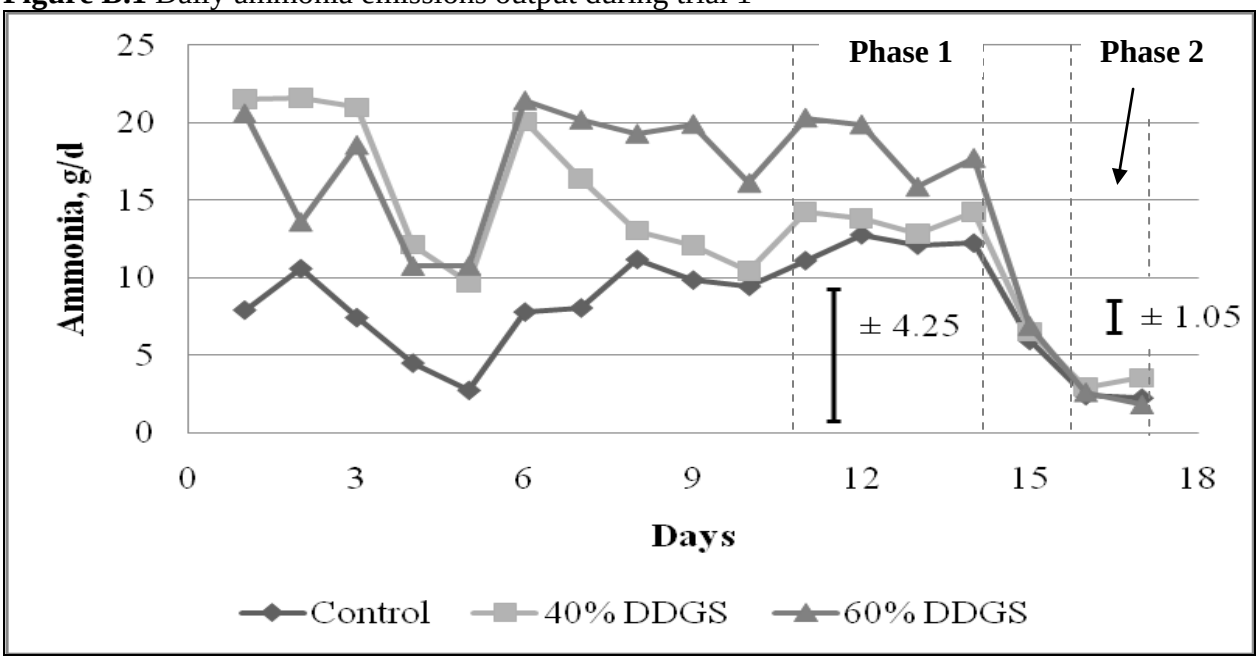
Trial 1

Table B.1 Comparison of unfiltered raw data to filtered data during trial 1

	Unfiltered raw data*				
Item	N	Mean	SD	Minimum	Maximum
Temperature, ºF	18547	58.863	3.944	49.108	68.569
Humidity, %	18547	49.663	11.234	24.896	104.000
Air flow, cfm	18547	500.968	188.588	0.000	654.083
Ammonia, ppm	18547	0.871	0.664	0.000	4.891
Nitric oxide, ppm	18547	0.017	0.014	0.012	0.216
Nitrogen dioxide, ppm	18547	0.064	0.028	0.017	0.270
Nitrous oxide, ppm	8064	0.650	0.251	0.096	1.313
	Filtered raw data*				
Item	N	Mean	SD	Minimum	Maximum
Temperature, ºF	18547	58.863	3.944	49.108	68.569
Humidity, %	18547	49.663	11.234	24.896	104.000
Air flow, cfm	17117	542.821	125.766	106.843	654.083
Ammonia, ppm	18547	0.871	0.664	0.000	4.891
Nitric oxide, ppm	18547	0.017	0.014	0.012	0.216
Nitrogen dioxide, ppm	18547	0.064	0.028	0.017	0.270
Nitrous oxide, ppm	8064	0.650	0.251	0.096	1.313

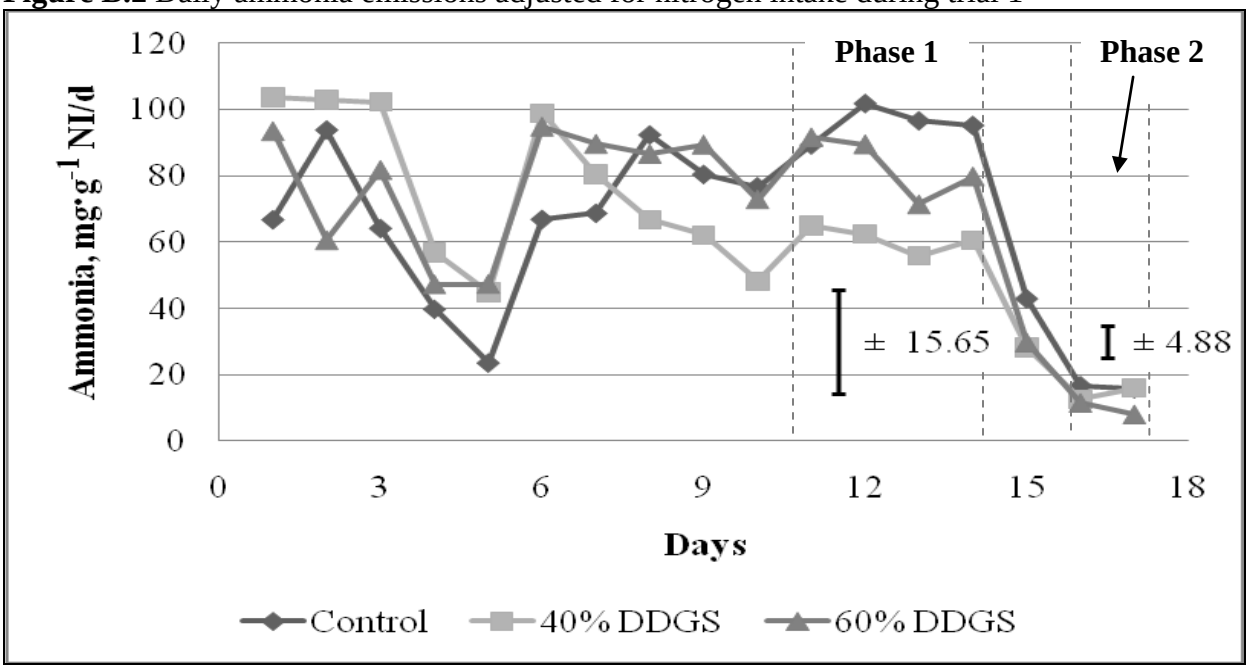
* Raw data represents all of the days cattle were housed within environmentally-controlled rooms

Figure B.1 Daily ammonia emissions output during trial 1



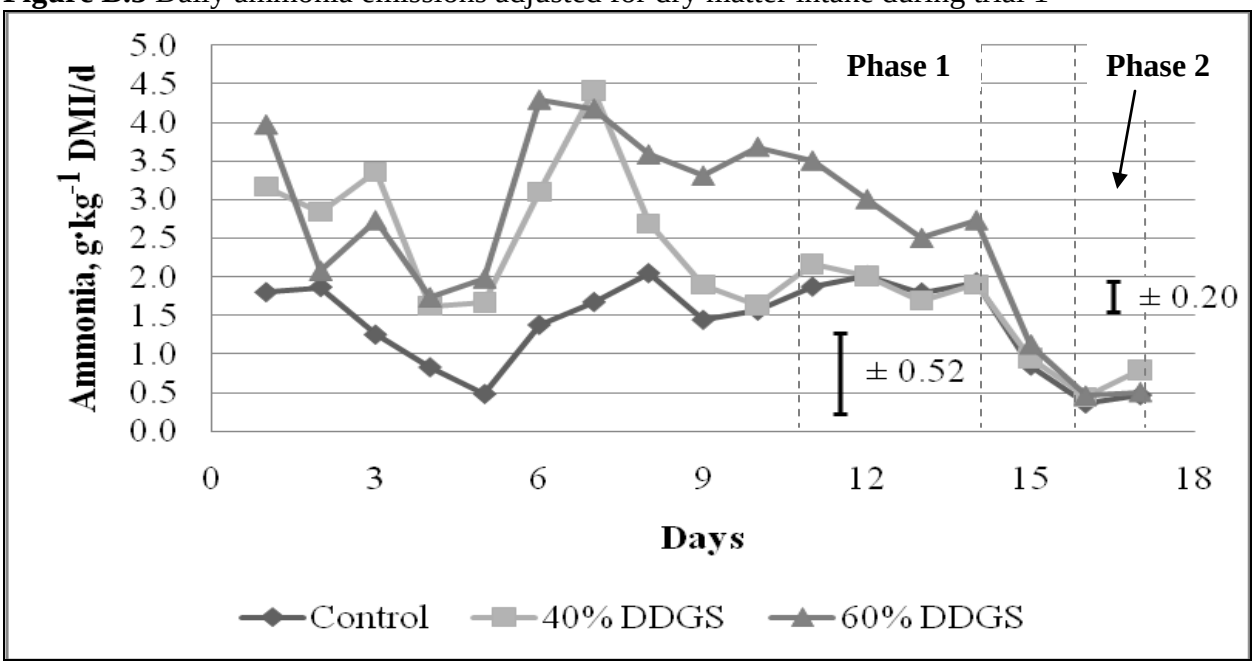
* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure B.2 Daily ammonia emissions adjusted for nitrogen intake during trial 1



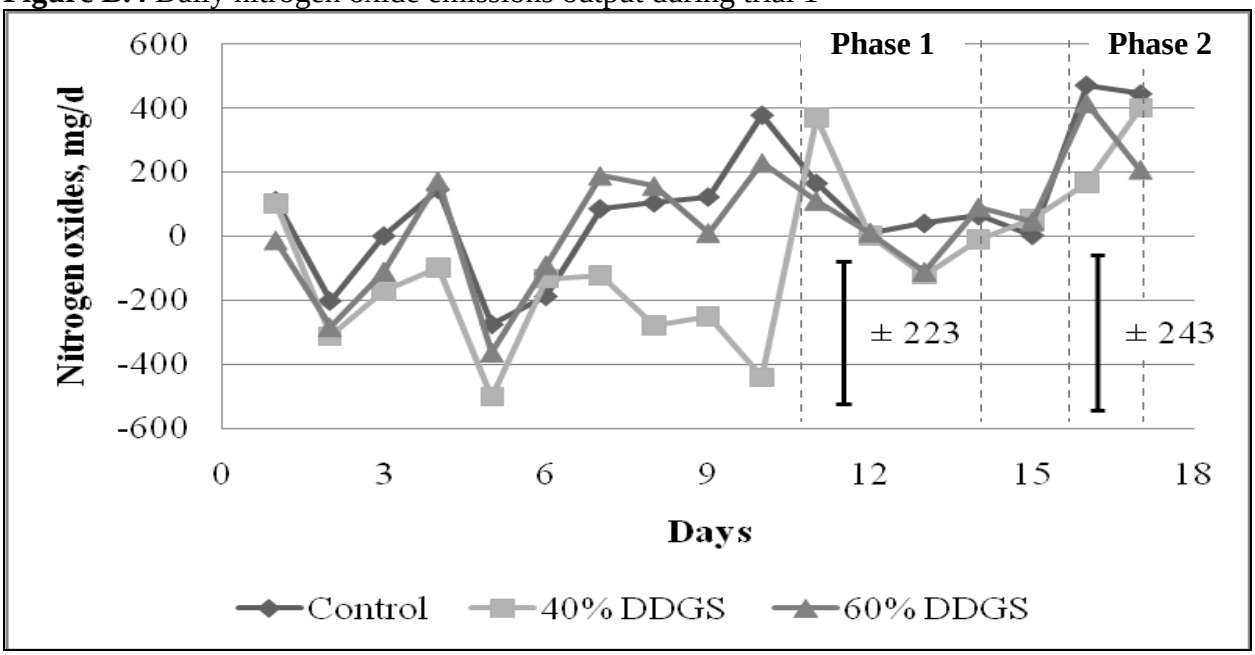
* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure B.3 Daily ammonia emissions adjusted for dry matter intake during trial 1



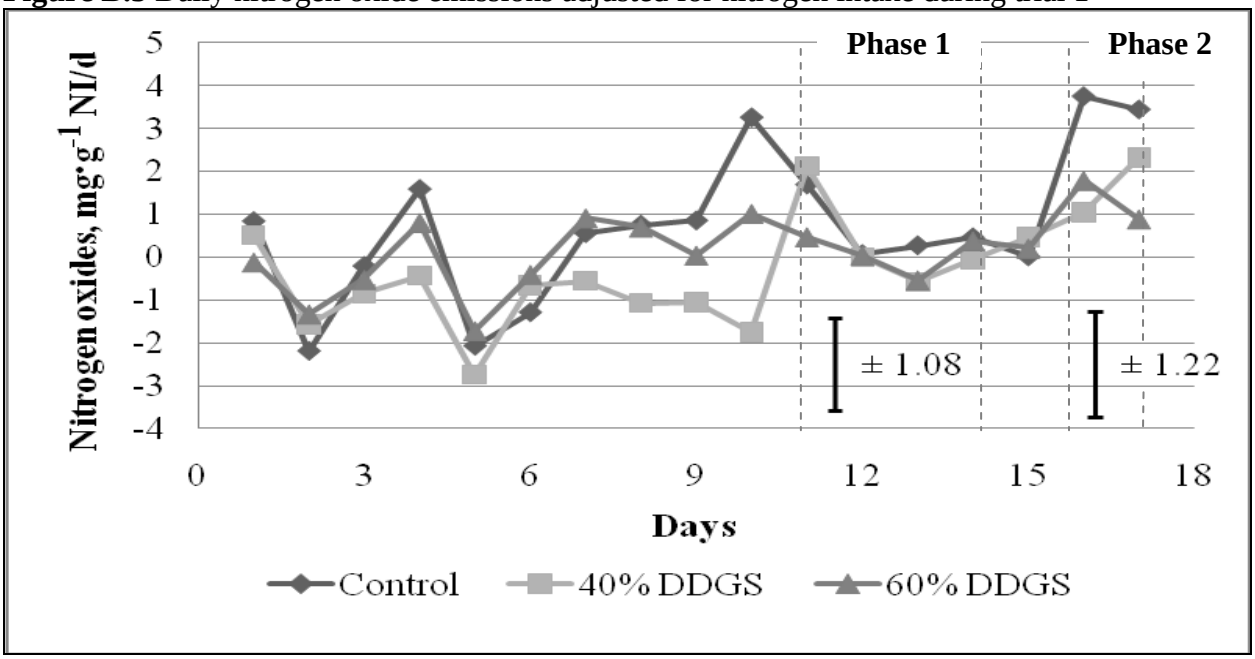
* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure B.4 Daily nitrogen oxide emissions output during trial 1



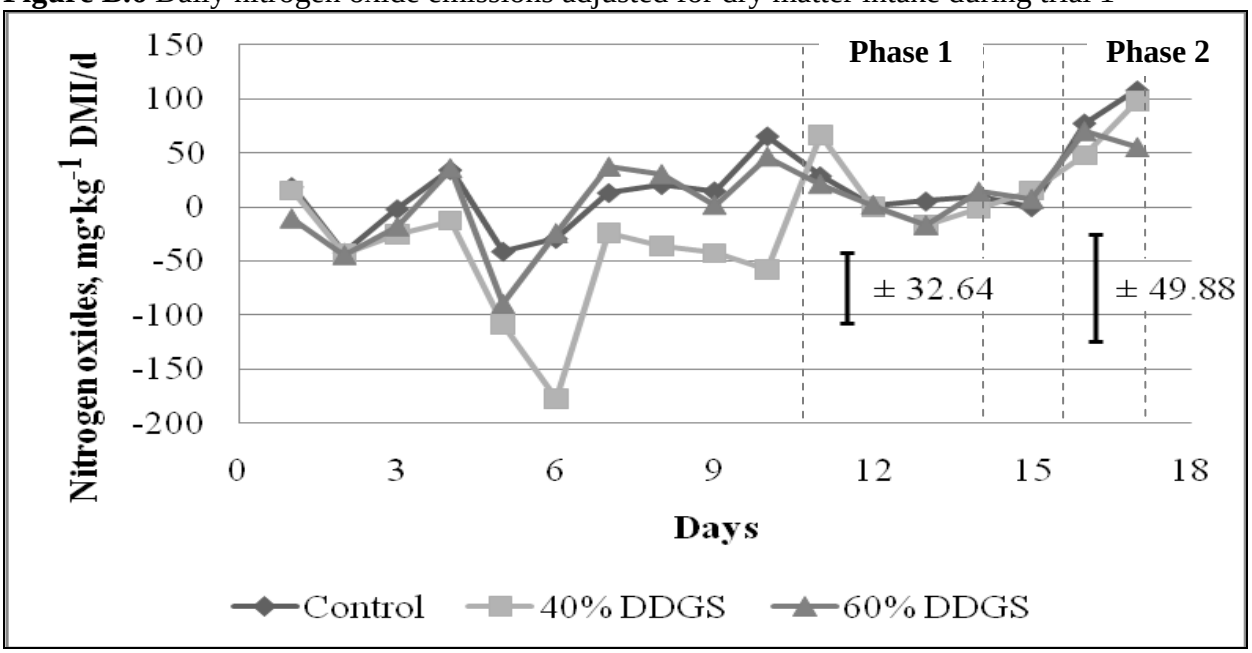
* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure B.5 Daily nitrogen oxide emissions adjusted for nitrogen intake during trial 1



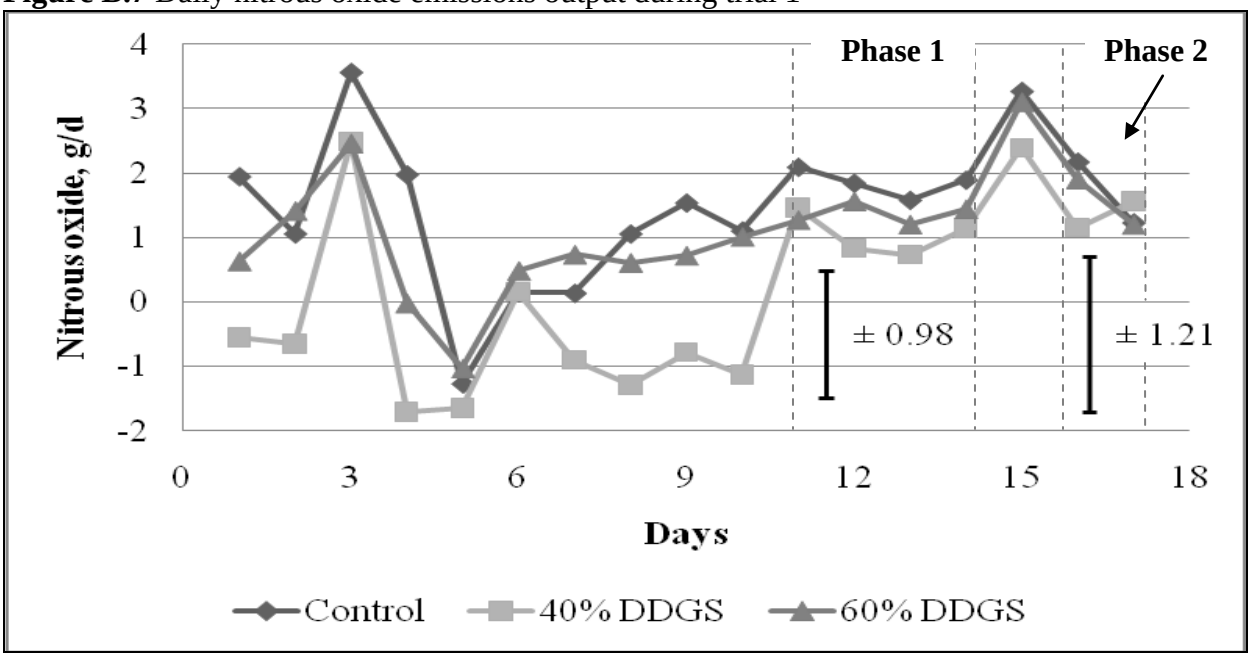
* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure B.6 Daily nitrogen oxide emissions adjusted for dry matter intake during trial 1



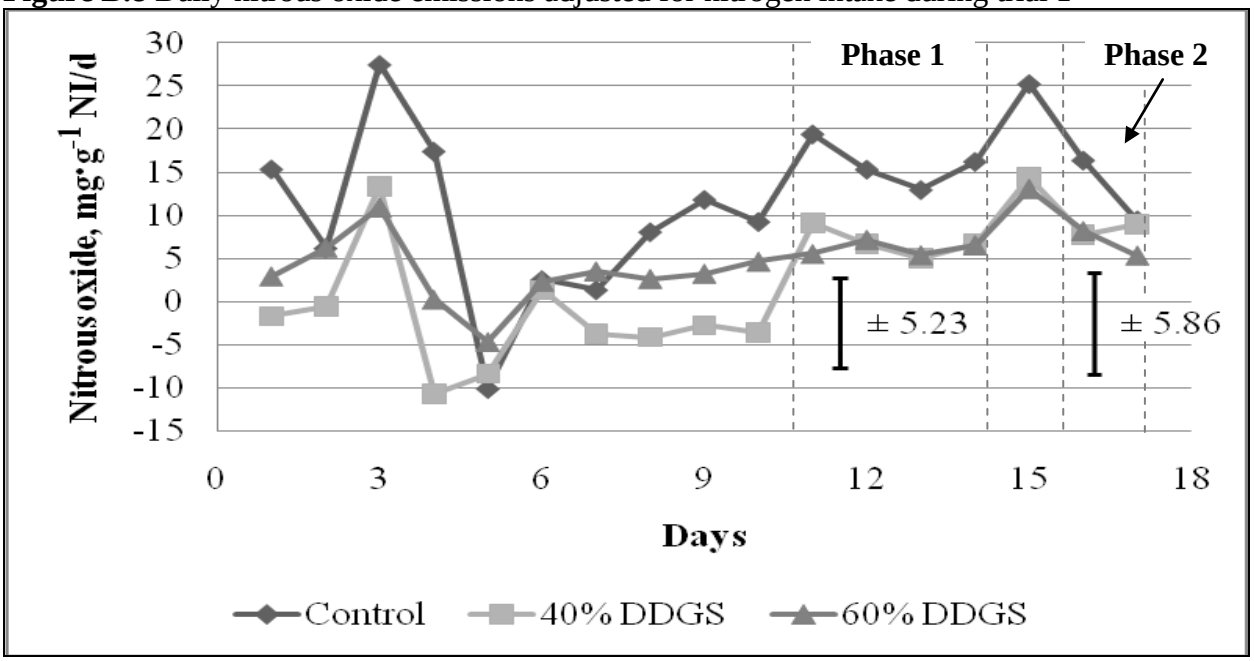
* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure B.7 Daily nitrous oxide emissions output during trial 1



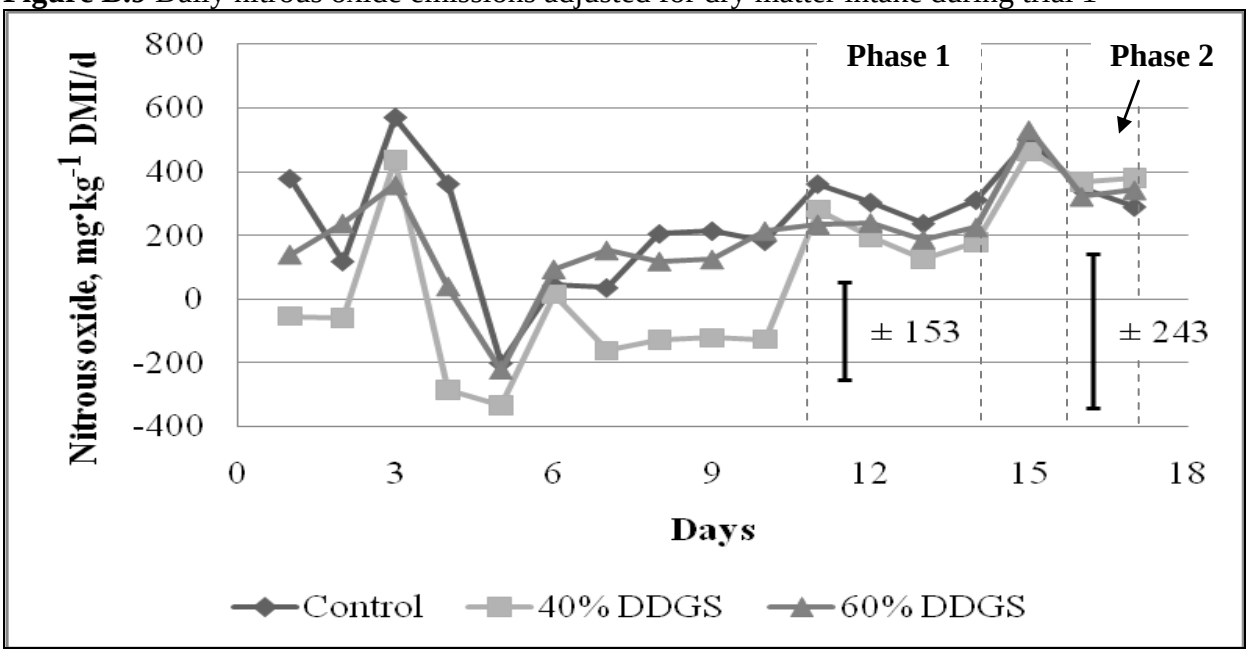
* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure B.8 Daily nitrous oxide emissions adjusted for nitrogen intake during trial 1



* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure B.9 Daily nitrous oxide emissions adjusted for dry matter intake during trial 1



* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Table B.2 Probability of day affects on nitrogenous emissions during trial 1

	Phase 1			Phase 2		
Item	TRT	Day	TRT × Day	TRT	Day	TRT × Day
Ammonia, g/d	0.66	0.13	0.22	0.80	0.73	0.23
Ammonia, mg/g NI	0.44	0.13	0.31	0.71	0.84	0.25
Ammonia, g/kg DMI	0.42	< 0.01	0.20	0.82	0.06	0.25
Nitrogen oxide, mg/d	0.49	< 0.01	0.05	0.33	0.79	0.22
Nitrogen oxide, mg/g NI	0.47	< 0.01	0.26	0.20	0.81	0.28
Nitrogen oxide, g/kg DMI	0.52	< 0.01	0.06	0.45	0.61	0.75
Nitrous oxide, g/d	0.29	0.42	0.75	0.40	0.86	0.18
Nitrous oxide, mg/g NI	0.18	0.24	0.72	0.37	0.57	0.15
Nitrous oxide, g/kg DMI	0.34	0.17	0.73	0.59	0.91	0.94

Figure B.10 Mean ammonia emissions output during trial 1

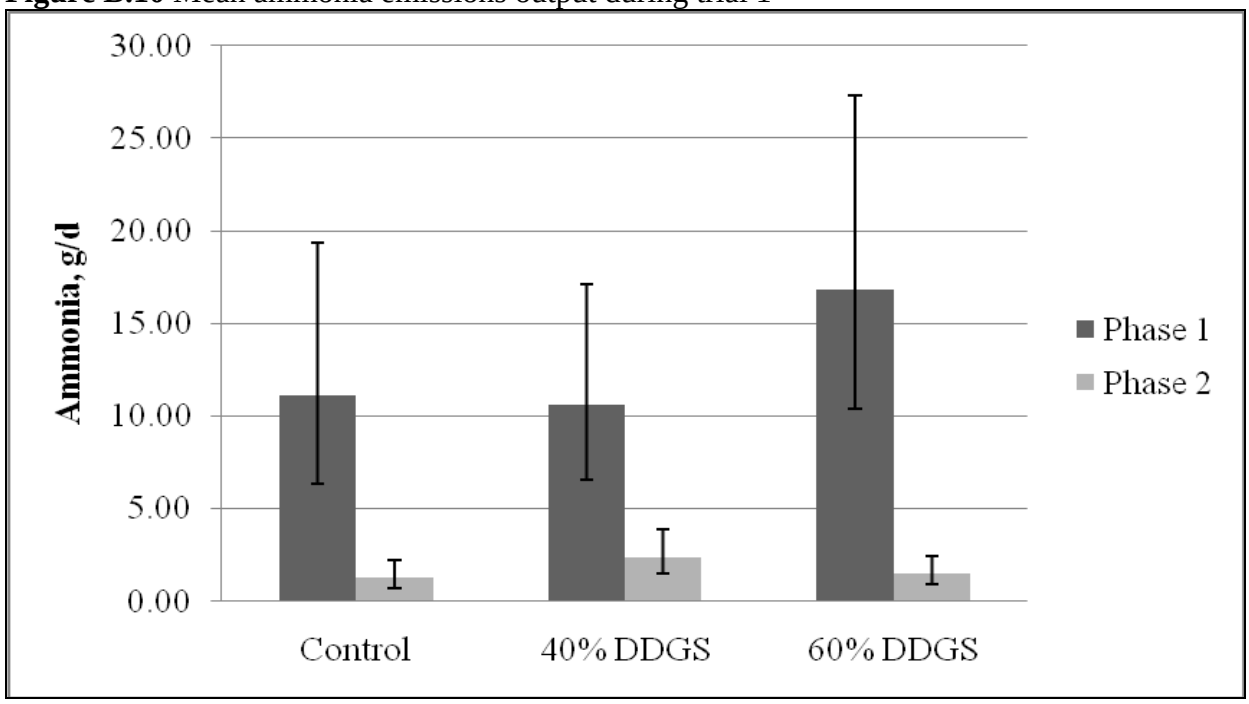


Figure B.11 Mean ammonia emissions adjusted for nitrogen intake during trial 1

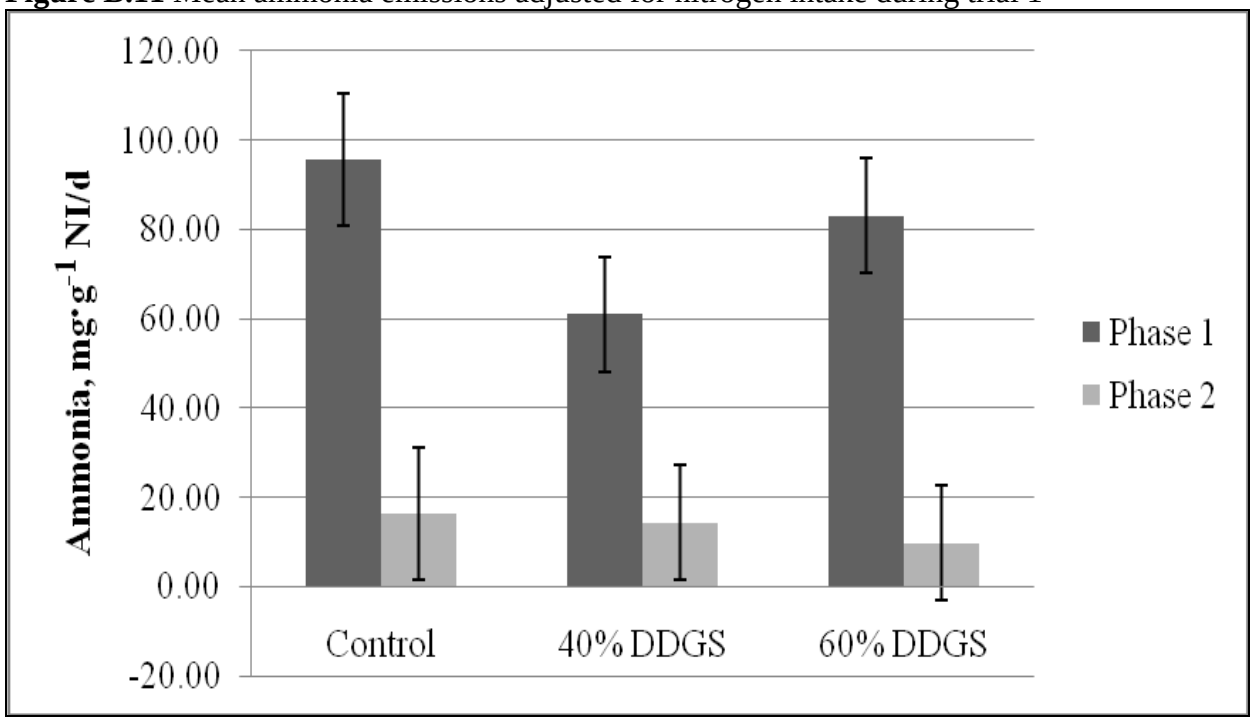


Figure B.12 Mean ammonia emissions adjusted for dry matter intake during trial 1

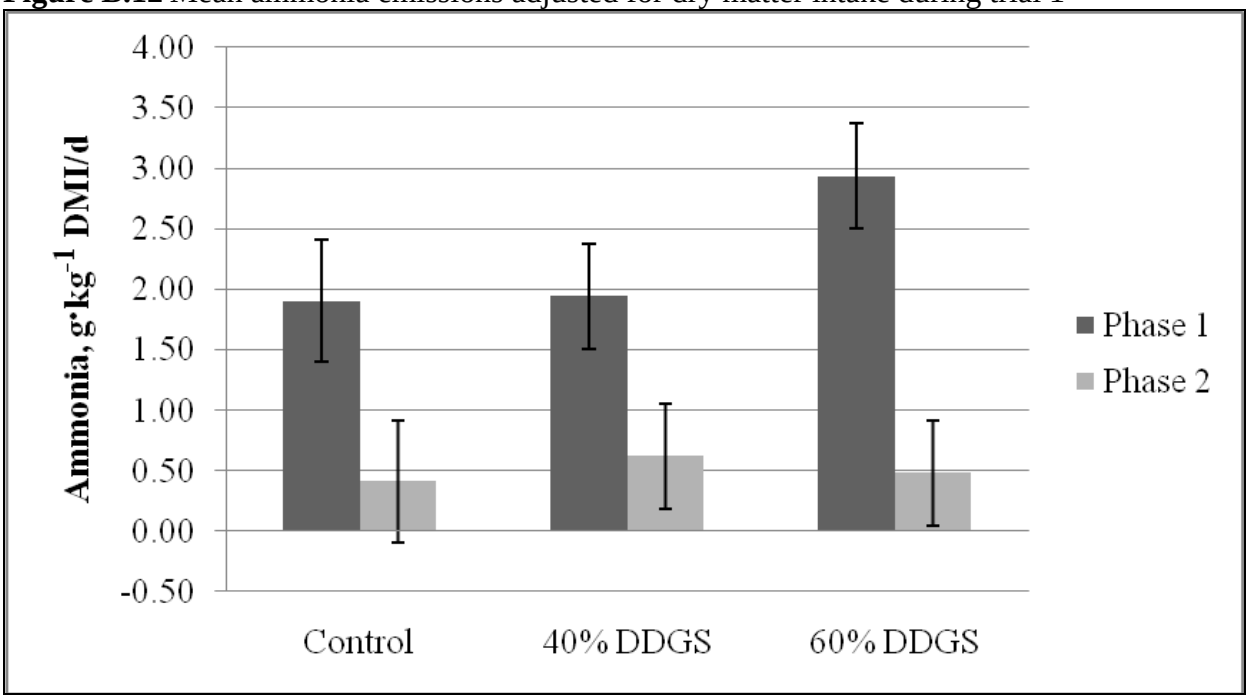
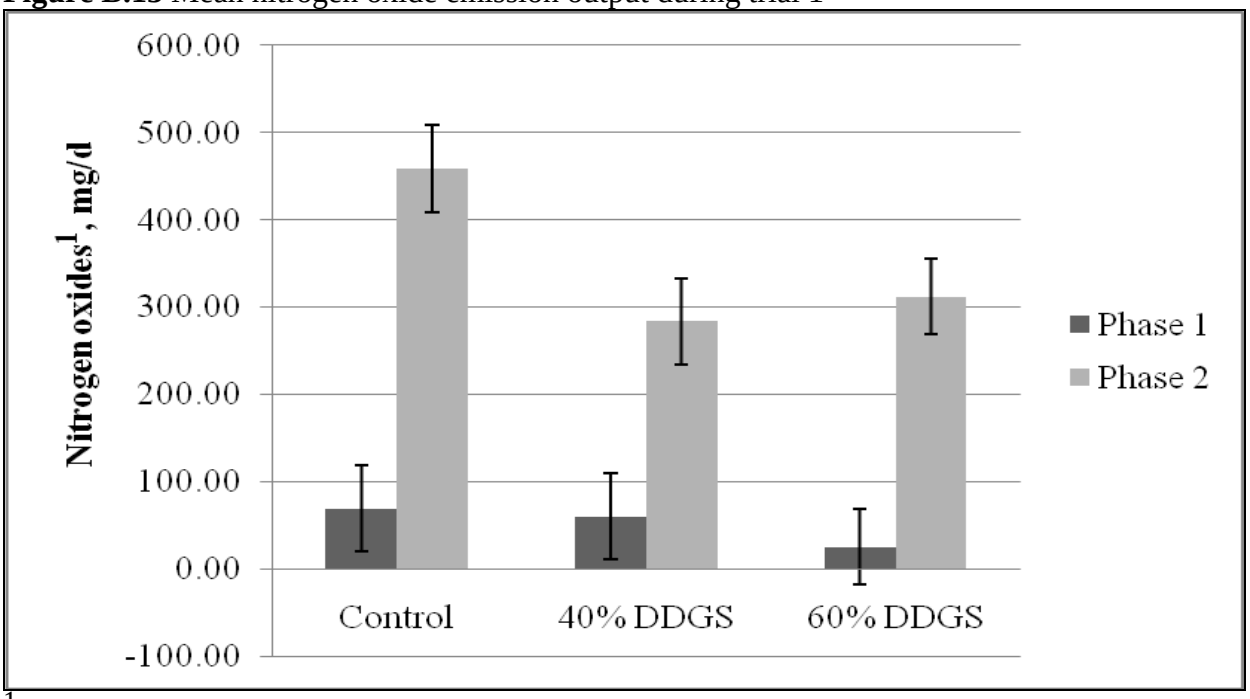
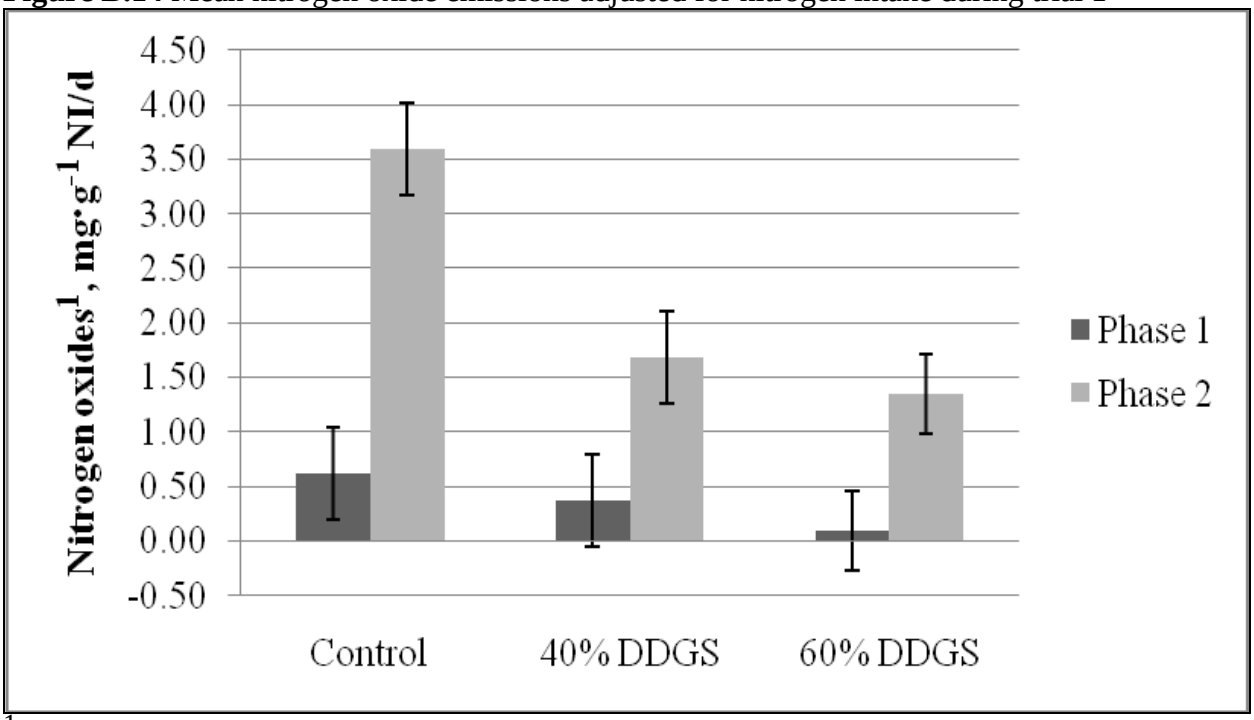


Figure B.13 Mean nitrogen oxide emission output during trial 1



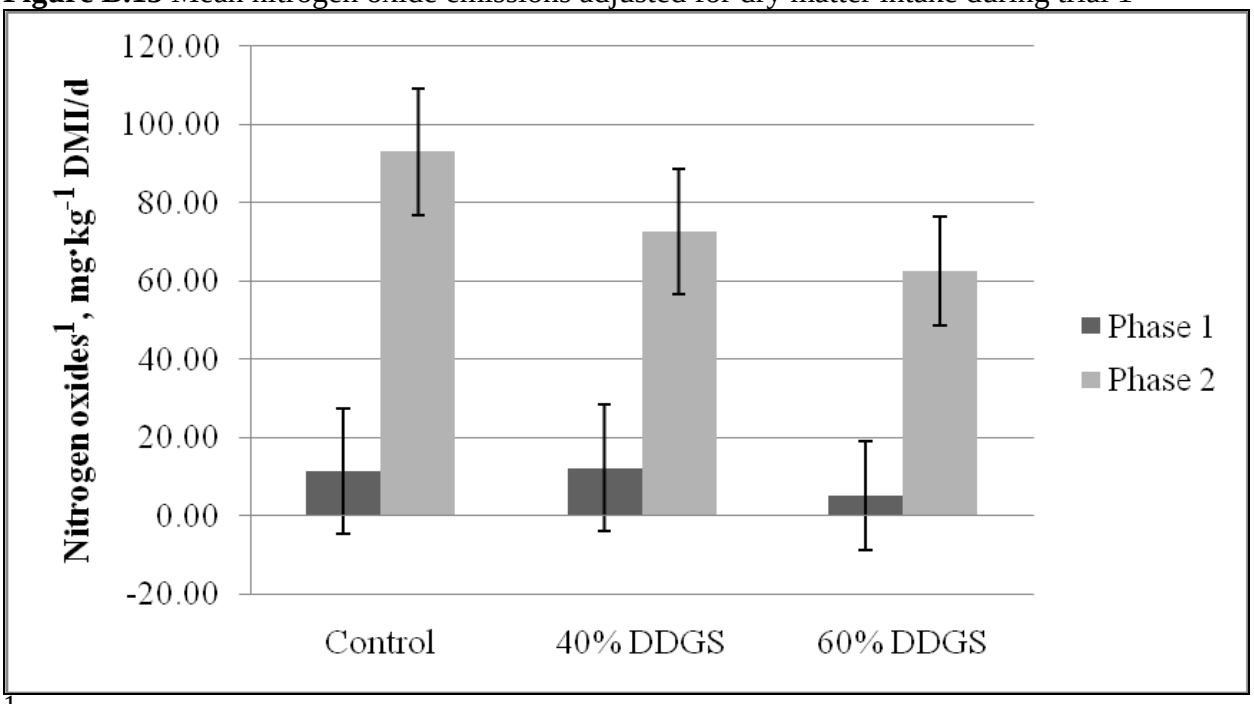
[1] Nitrogen oxide includes both NO^- and NO_2 gas

Figure B.14 Mean nitrogen oxide emissions adjusted for nitrogen intake during trial 1



[1] Nitrogen oxide includes both NO^- and NO_2 gas

Figure B.15 Mean nitrogen oxide emissions adjusted for dry matter intake during trial 1



[1] Nitrogen oxide includes both NO^- and NO_2 gas

Figure B.16 Mean nitrous oxide emissions output during trial 1

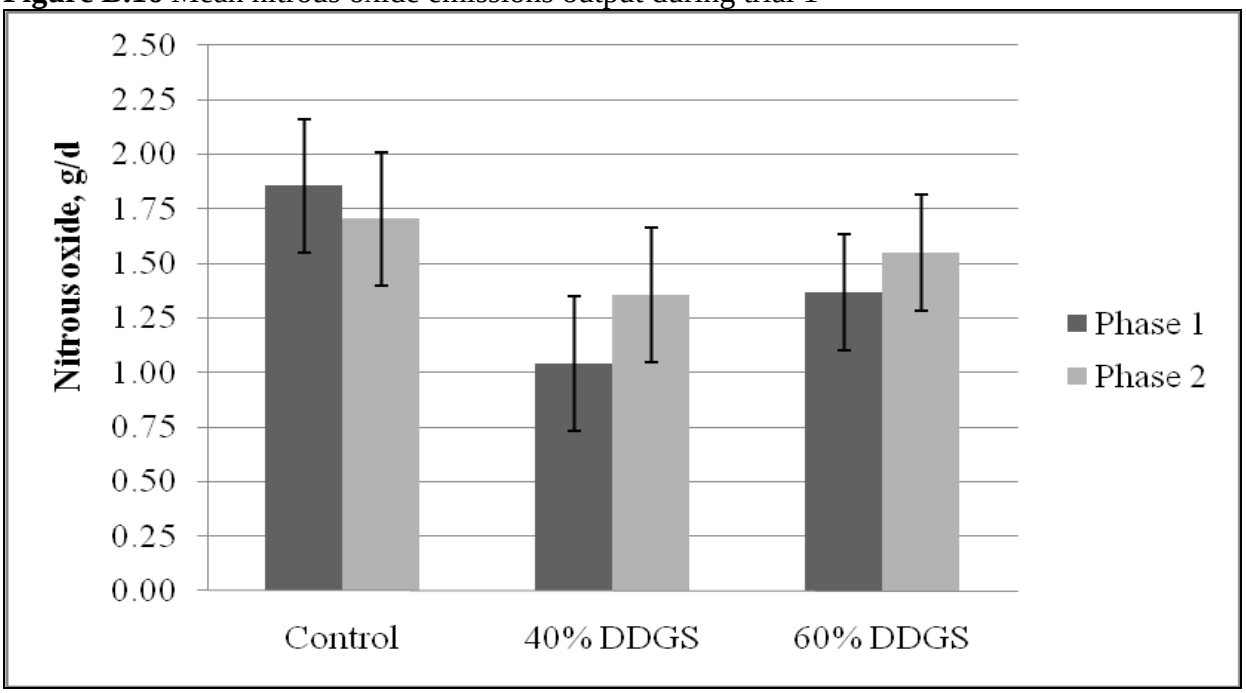


Figure B.17 Mean nitrous oxide emissions adjusted for nitrogen intake during trial 1

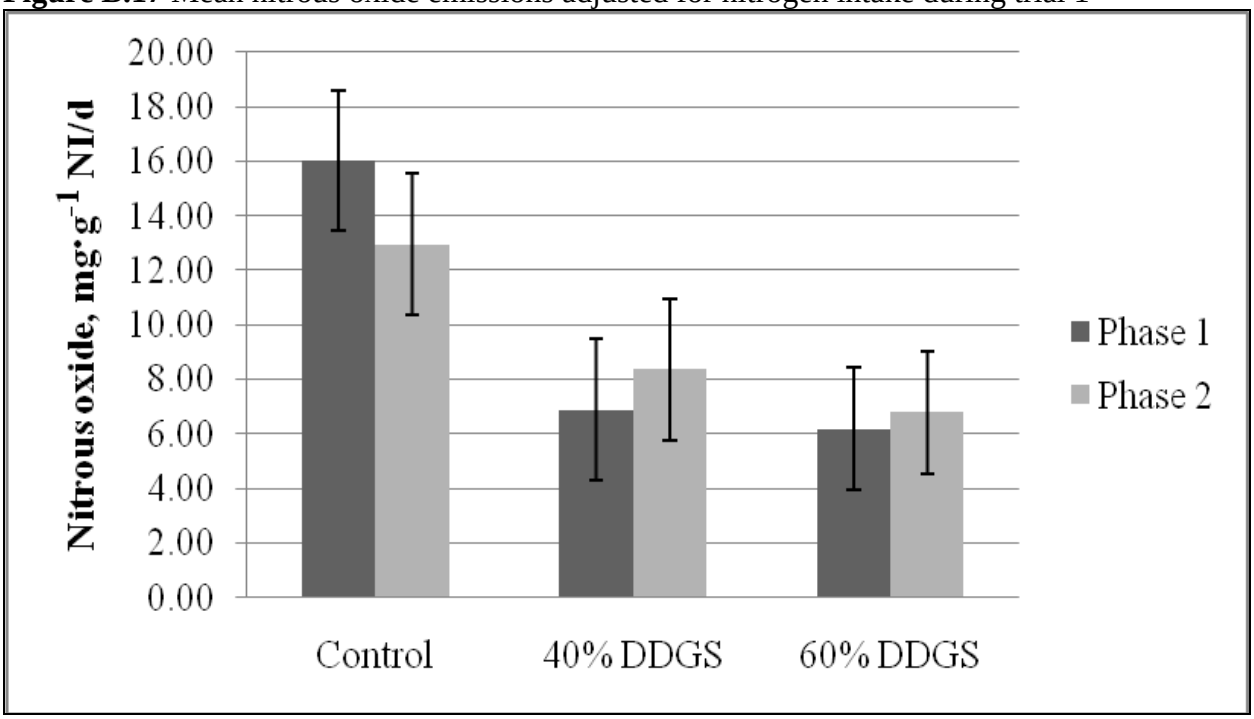
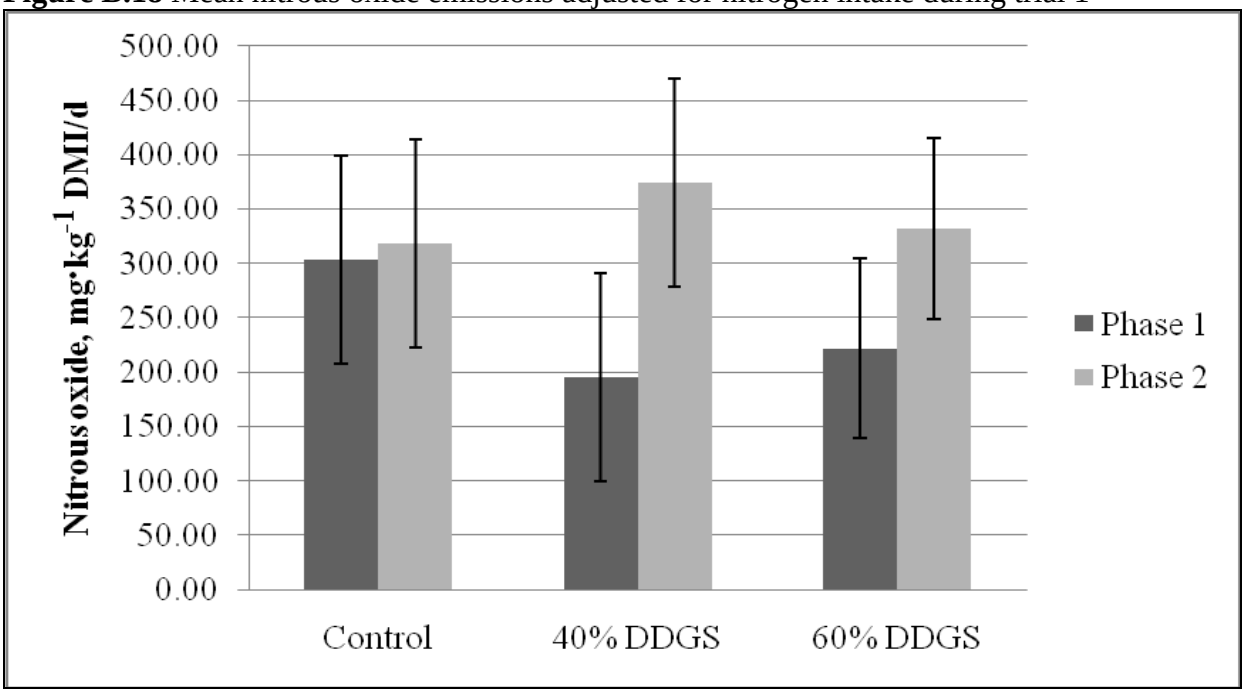


Figure B.18 Mean nitrous oxide emissions adjusted for nitrogen intake during trial 1



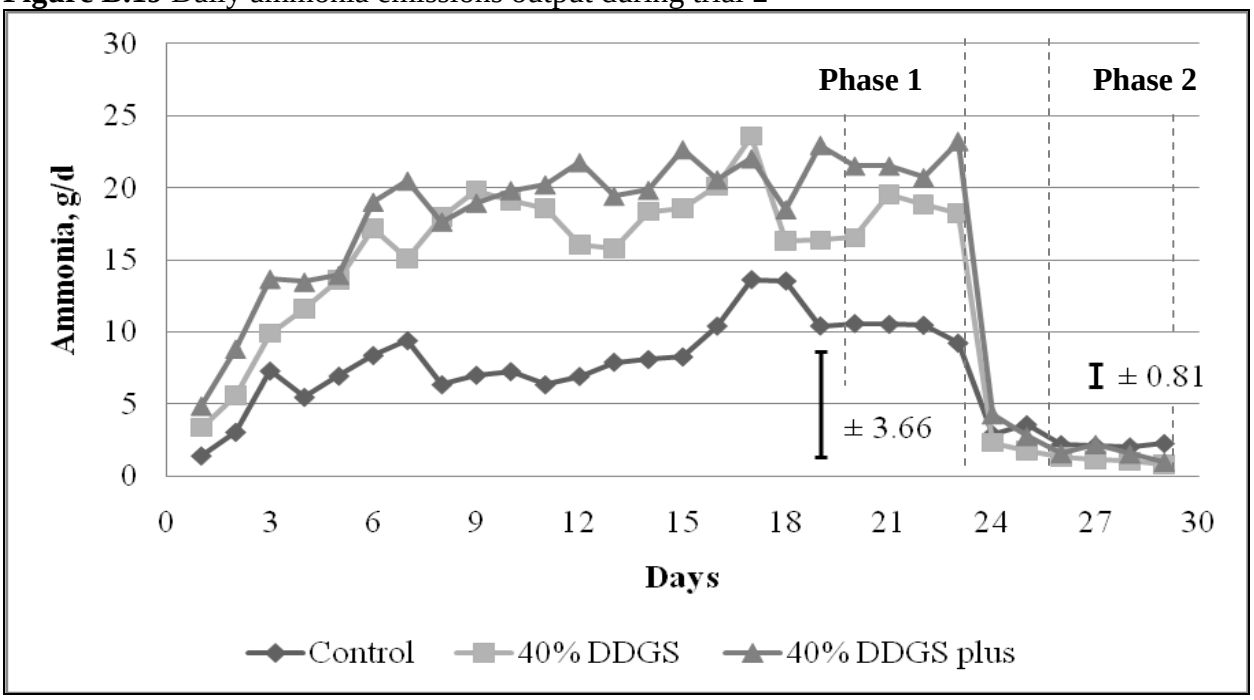
Trial 2

Table B.3 Comparison of unfiltered raw data to filtered data during trial 2

	Unfiltered raw data*				
Item	N	Mean	SD	Minimum	Maximum
Temperature, ºF	28870	60.367	1.813	48.688	65.353
Humidity, %	28870	36.041	11.308	16.109	104.000
Air flow, cfm	28870	545.111	179.405	0.000	659.829
Ammonia, ppm	28870	0.870	0.575	-0.017	5.771
Nitric oxide, ppm	28870	0.086	0.072	-0.020	3.540
Nitrogen dioxide, ppm	28870	0.131	0.118	-0.026	6.684
Nitrous oxide, ppm	12897	-0.019	0.064	-0.253	0.238
	Filtered raw data*				
Item	N	Mean	SD	Minimum	Maximum
Temperature, ºF	28870	60.367	1.813	48.688	65.353
Humidity, %	28870	36.041	11.308	16.109	104.000
Air flow, cfm	26609	591.430	86.750	97.005	659.829
Ammonia, ppm	28870	0.870	0.575	-0.017	5.771
Nitric oxide, ppm	28860	0.086	0.061	-0.020	0.335
Nitrogen dioxide, ppm	28853	0.129	0.047	-0.026	0.344
Nitrous oxide, ppm	12897	-0.019	0.064	-0.253	0.238

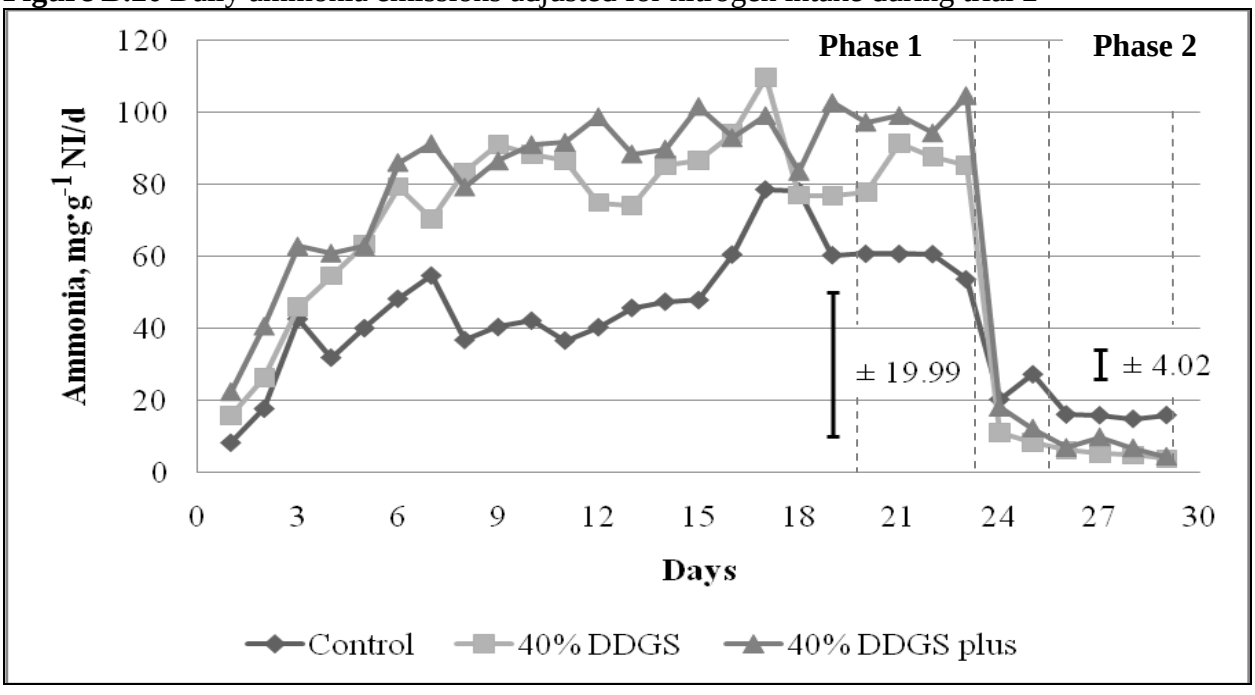
* Raw data represents all of the days cattle were housed within environmentally-controlled rooms

Figure B.19 Daily ammonia emissions output during trial 2



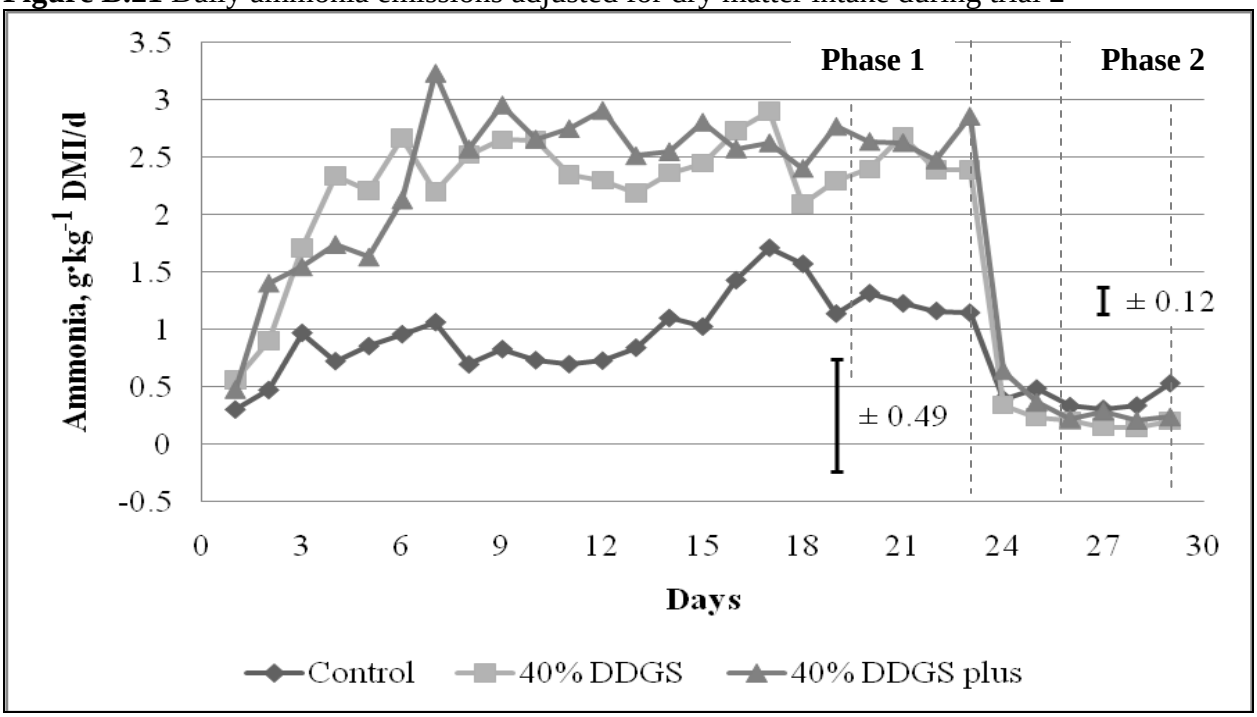
* Days 20-23 represent steady state mean emissions during phase 1. Days 26-29 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure B.20 Daily ammonia emissions adjusted for nitrogen intake during trial 2



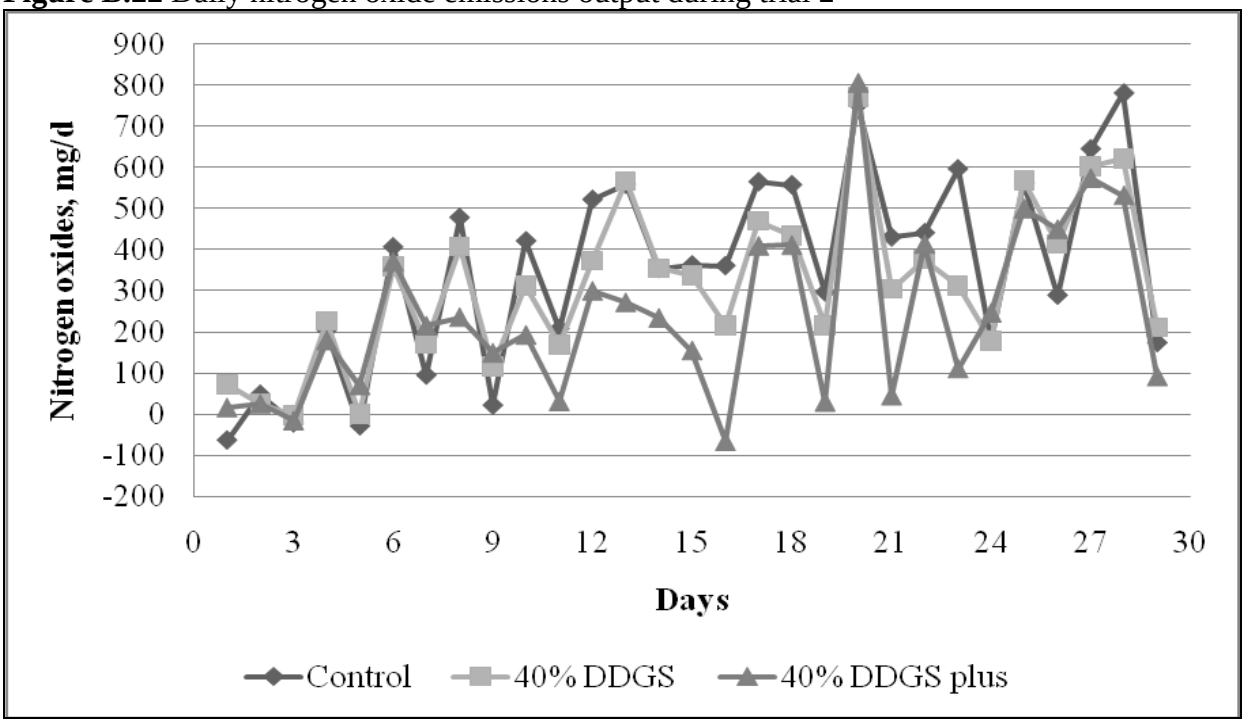
* Days 20-23 represent steady state mean emissions during phase 1. Days 26-29 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure B.21 Daily ammonia emissions adjusted for dry matter intake during trial 2



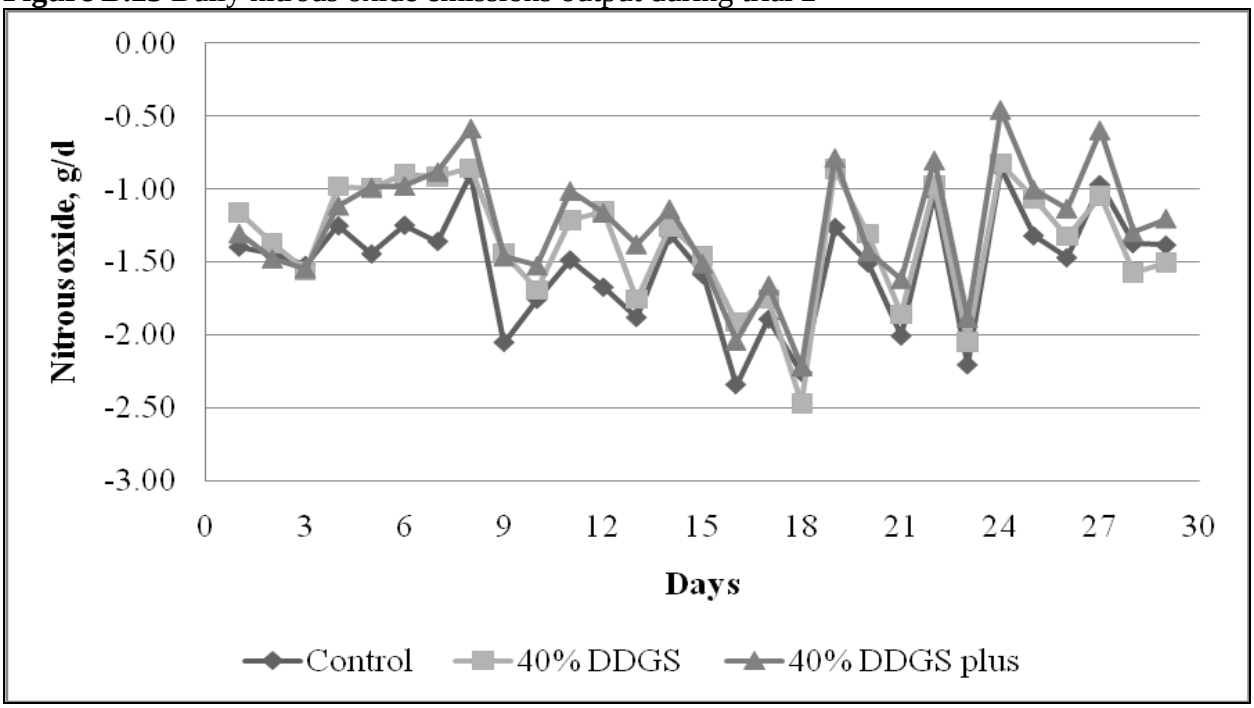
* Days 20-23 represent steady state mean emissions during phase 1. Days 26-29 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure B.22 Daily nitrogen oxide emissions output during trial 2



* Days 20-23 represent mean emissions during phase 1. Days 26-29 represent means for phase 2

Figure B.23 Daily nitrous oxide emissions output during trial 2



* Days 20-23 represent mean emissions during phase 1. Days 26-29 represent means for phase 2

Figure B.23 Mean ammonia emission output during trial 2

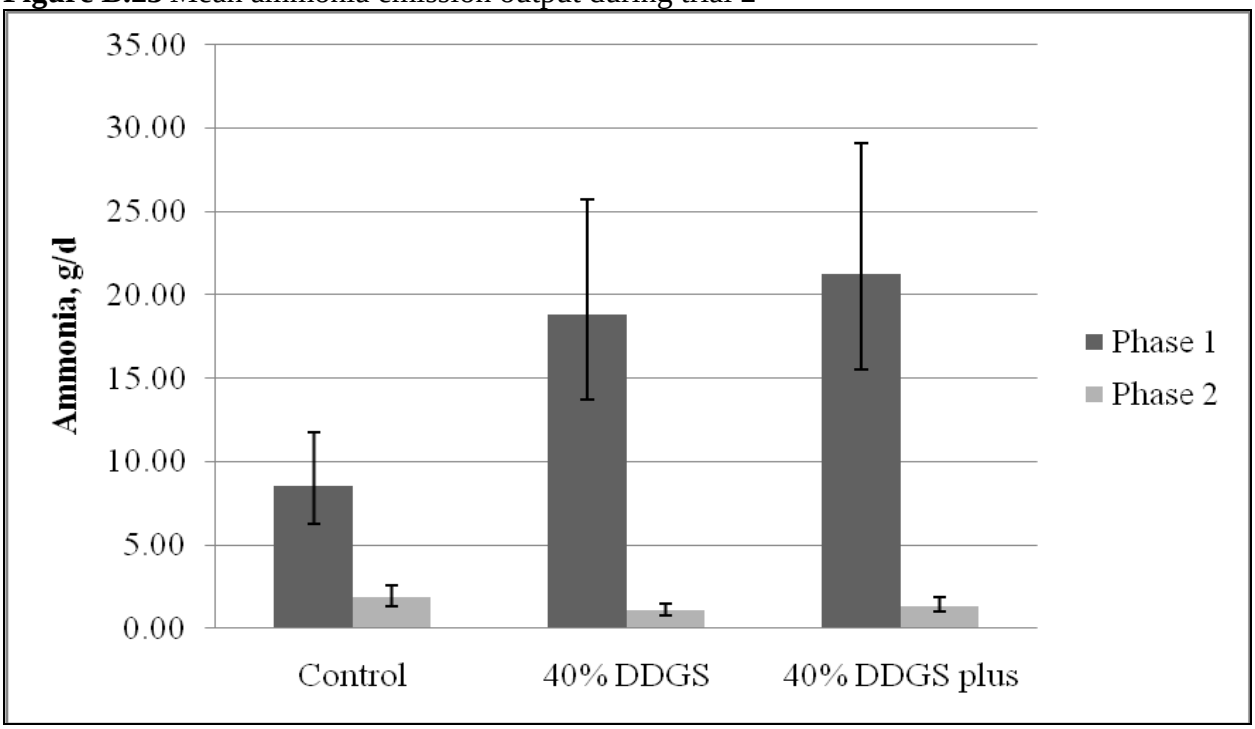


Figure B.24 Mean ammonia emissions adjusted for nitrogen intake during trial 2

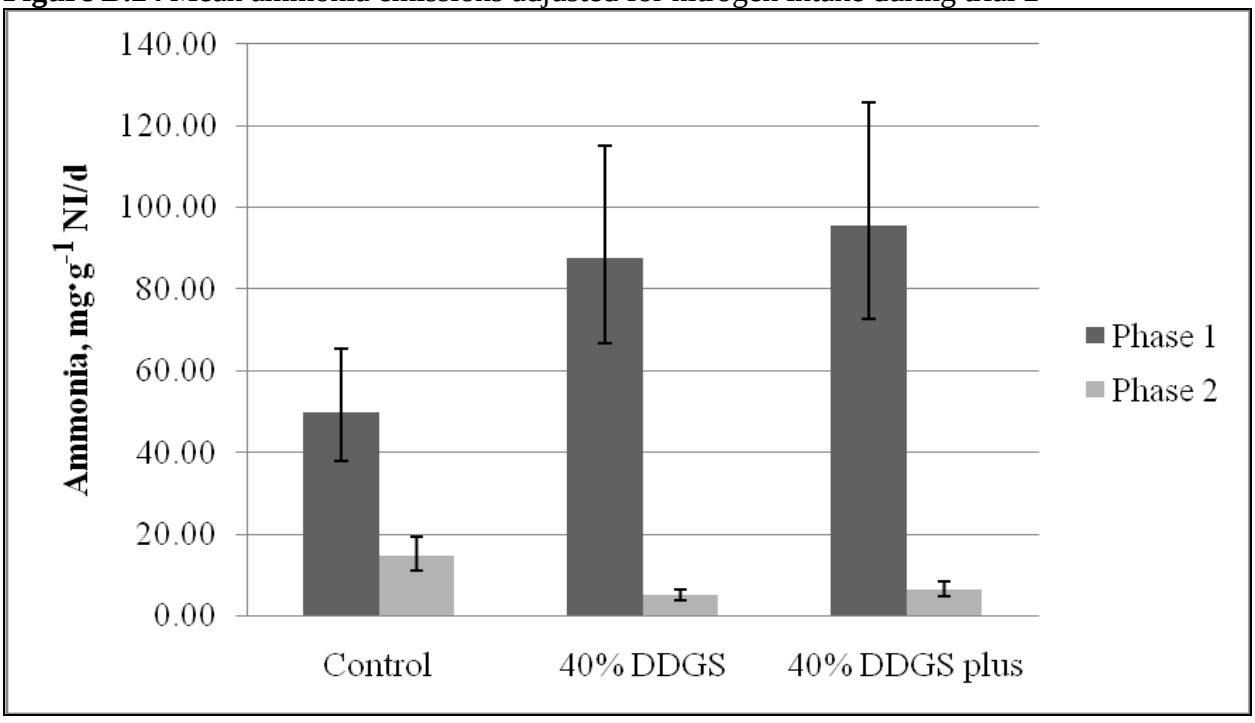


Figure B.25 Mean ammonia emissions adjusted for dry matter intake during trial 2

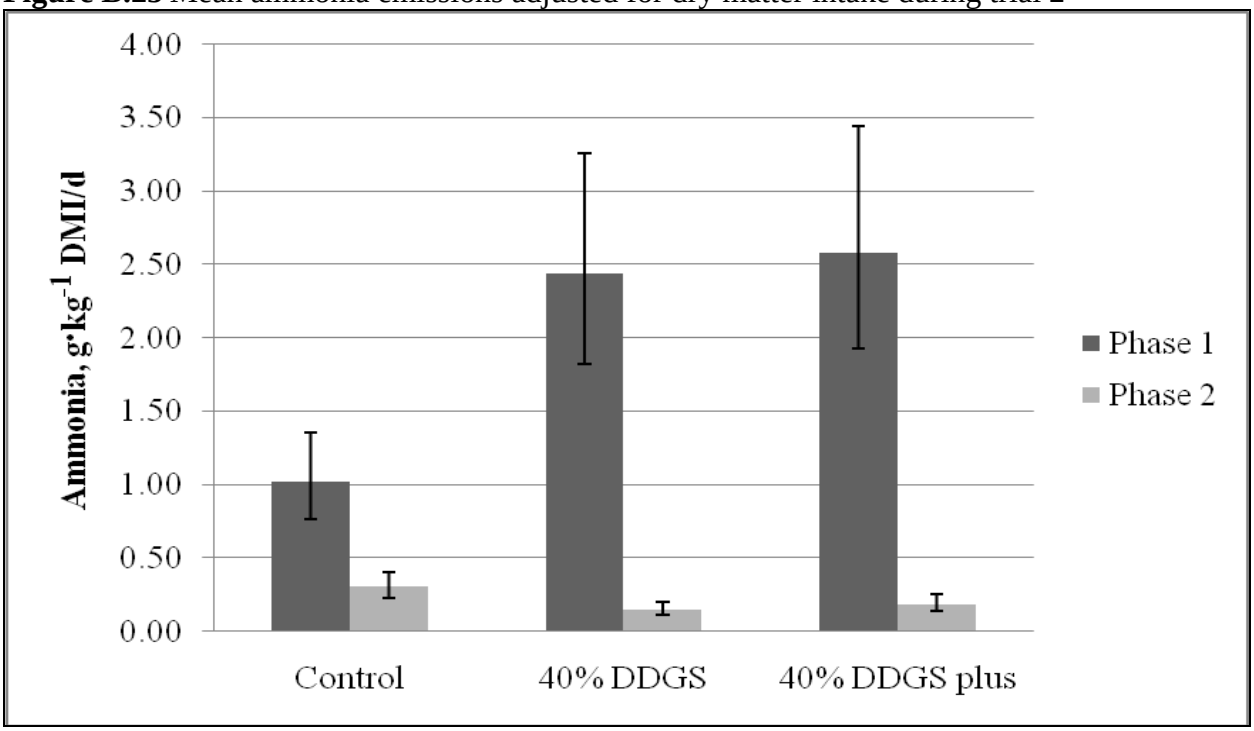


Figure B.26 Mean nitrogen oxide emissions output during trial 2

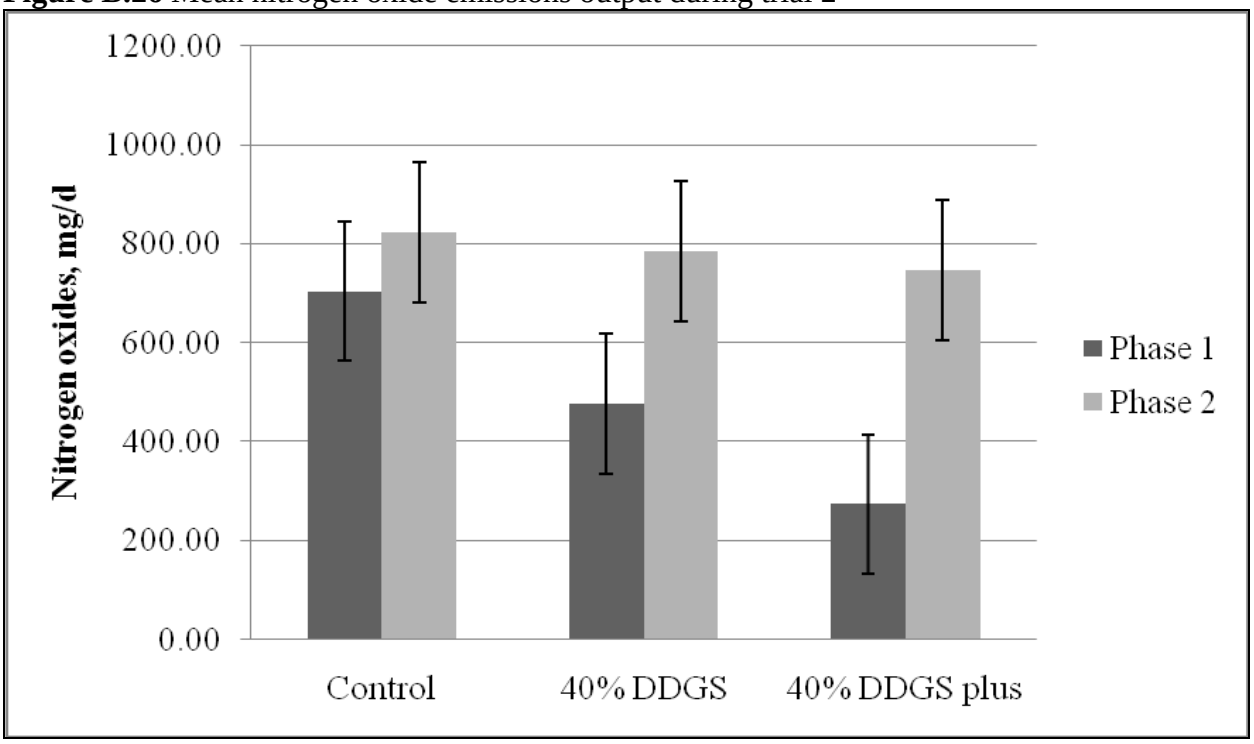


Figure B.27 Mean nitrogen oxide emissions adjusted for nitrogen intake during trial 2

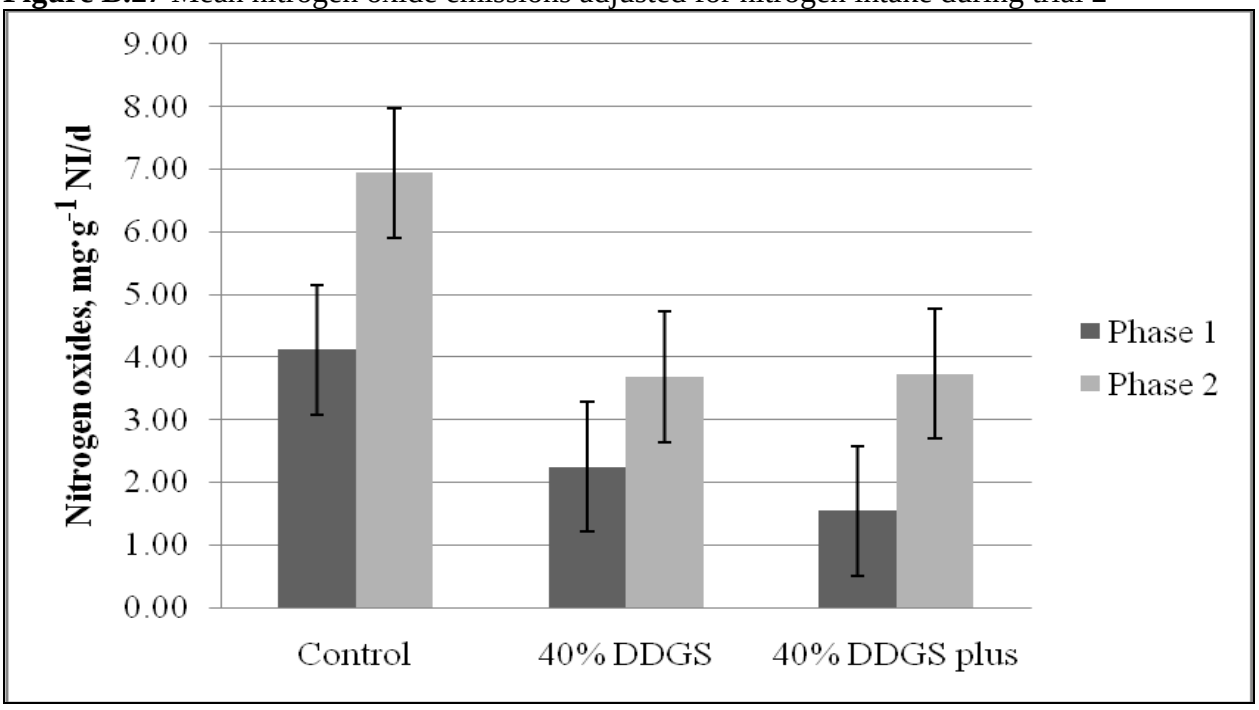


Figure B.28 Mean nitrogen oxide emissions adjusted for dry matter intake during trial 2

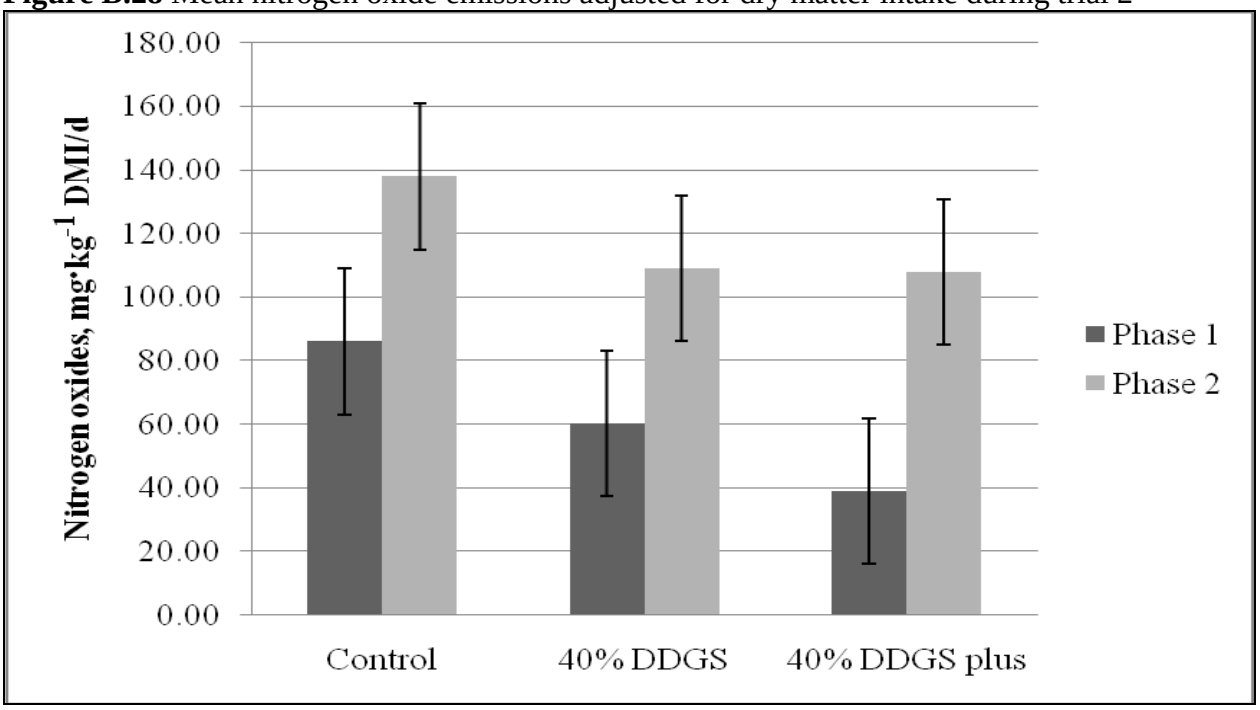


Figure B.29 Mean nitrous oxide emissions output during trial 2

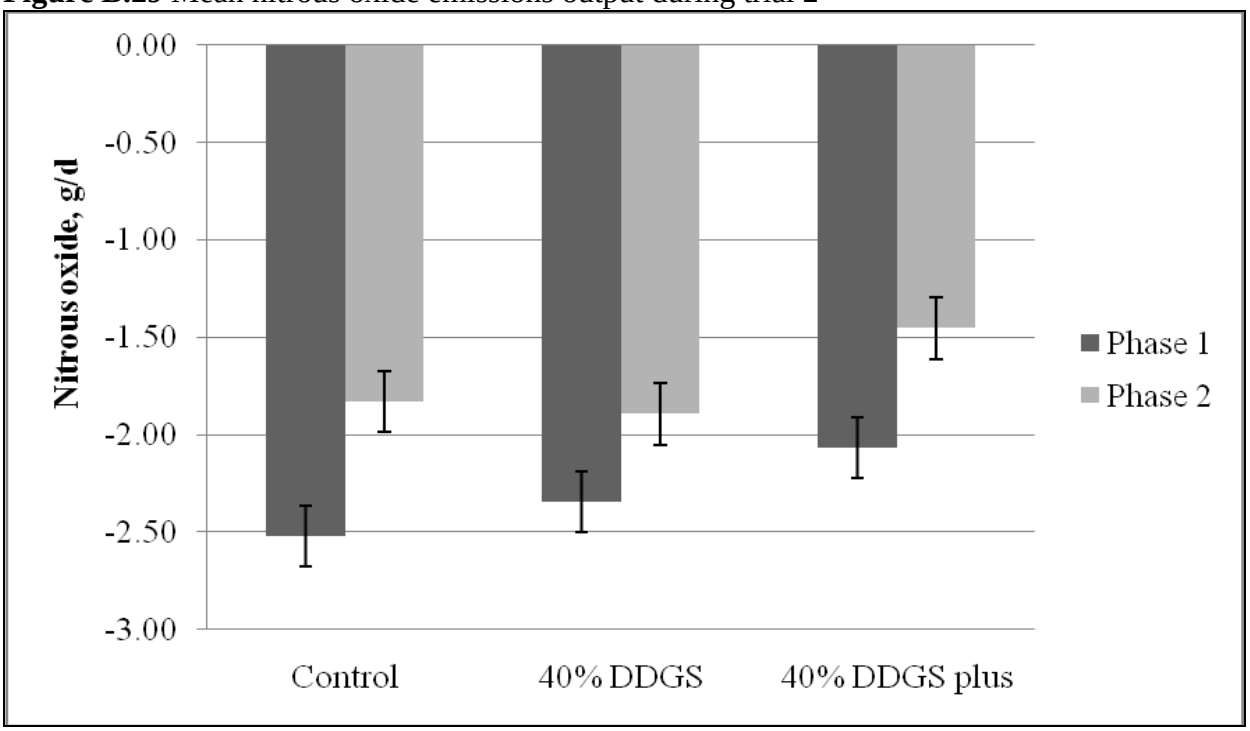


Figure B.30 Mean nitrous oxide emissions adjusted for nitrogen intake during trial 2

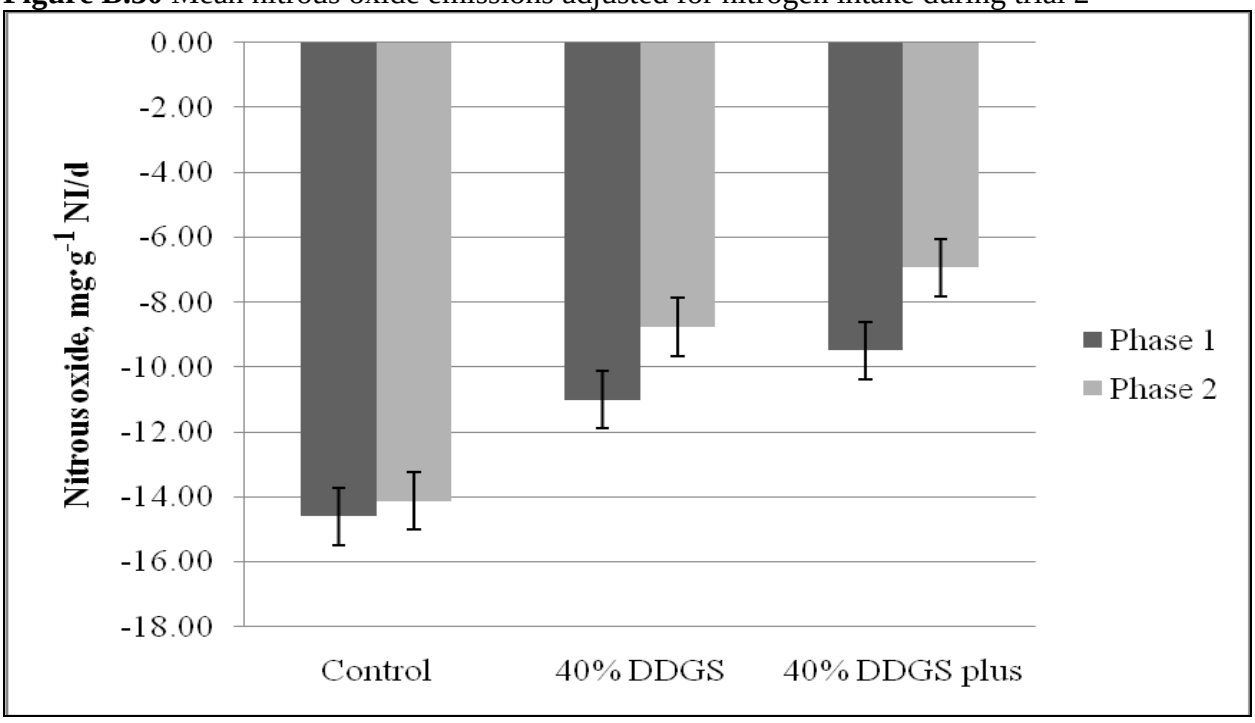
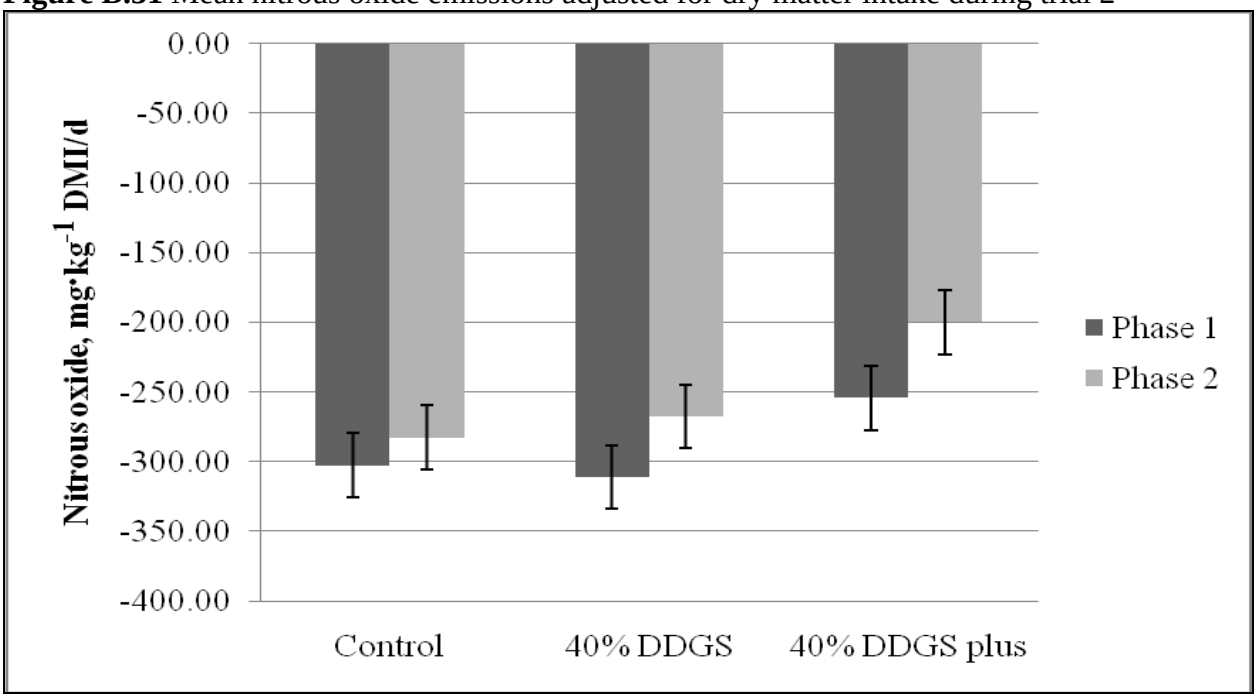


Figure B.31 Mean nitrous oxide emissions adjusted for dry matter intake during trial 2



APPENDIX C

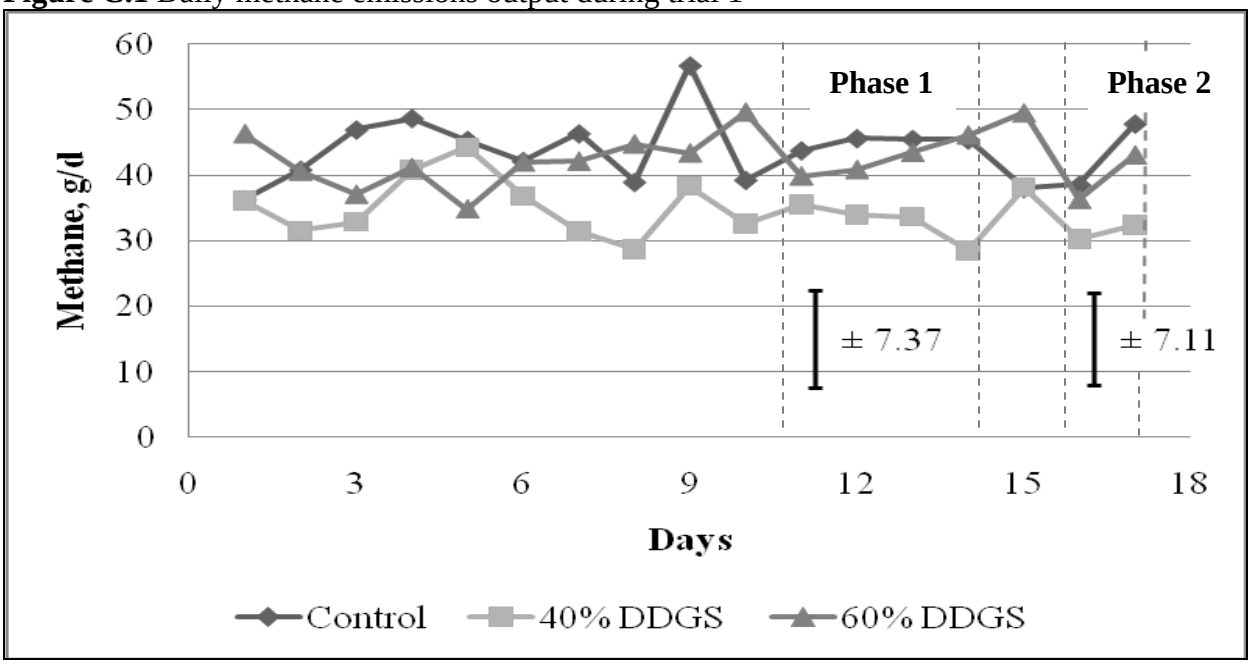
Trial 1

Table C.1 Comparison of unfiltered raw data to filtered data during trial 1

	Unfiltered raw data				
Variable	N	Mean	SD	Minimum	Maximum
Temperature,º F	18547	58.863	3.944	49.108	68.569
Humidity, %	18547	49.663	11.234	24.896	104.000
Air flow, cfm	18547	500.968	188.588	0.000	654.083
CH_4, ppm	18547	5.111	2.057	2.056	29.609
NMTHC, ppm	18547	0.055	0.038	0.000	0.291
CO_2, ppm	18547	619.836	99.749	392.140	1355.350
O_2, %	18547	20.811	0.199	20.627	26.545

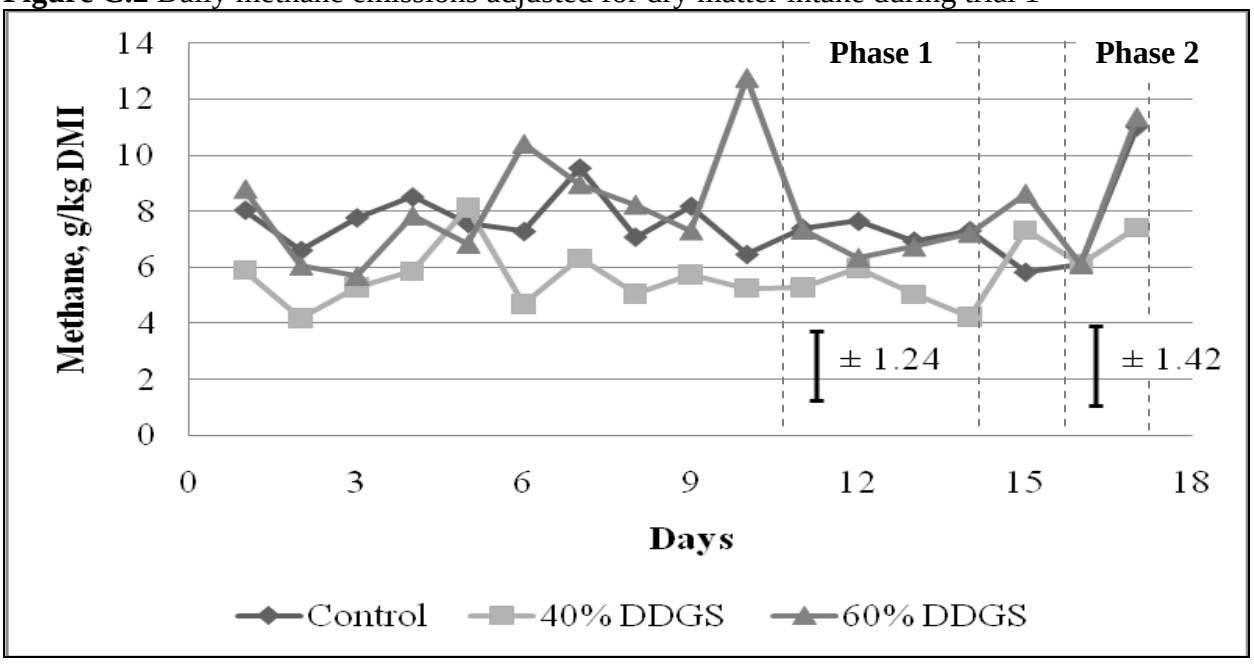
	Filtered raw data				
Variable	N	Mean	SD	Minimum	Maximum
Temperature,º F	18547	58.863	3.944	49.108	68.569
Humidity, %	18547	49.663	11.234	24.896	104.000
Air flow, cfm	17117	542.821	125.766	106.843	654.083
CH_4, ppm	18547	5.111	2.057	2.056	29.609
NMTHC, ppm	18547	0.055	0.038	0.000	0.291
CO_2, ppm	18520	618.899	96.740	392.140	1198.020
O_2, %	18518	20.804	0.063	20.627	21.387

Figure C.1 Daily methane emissions output during trial 1



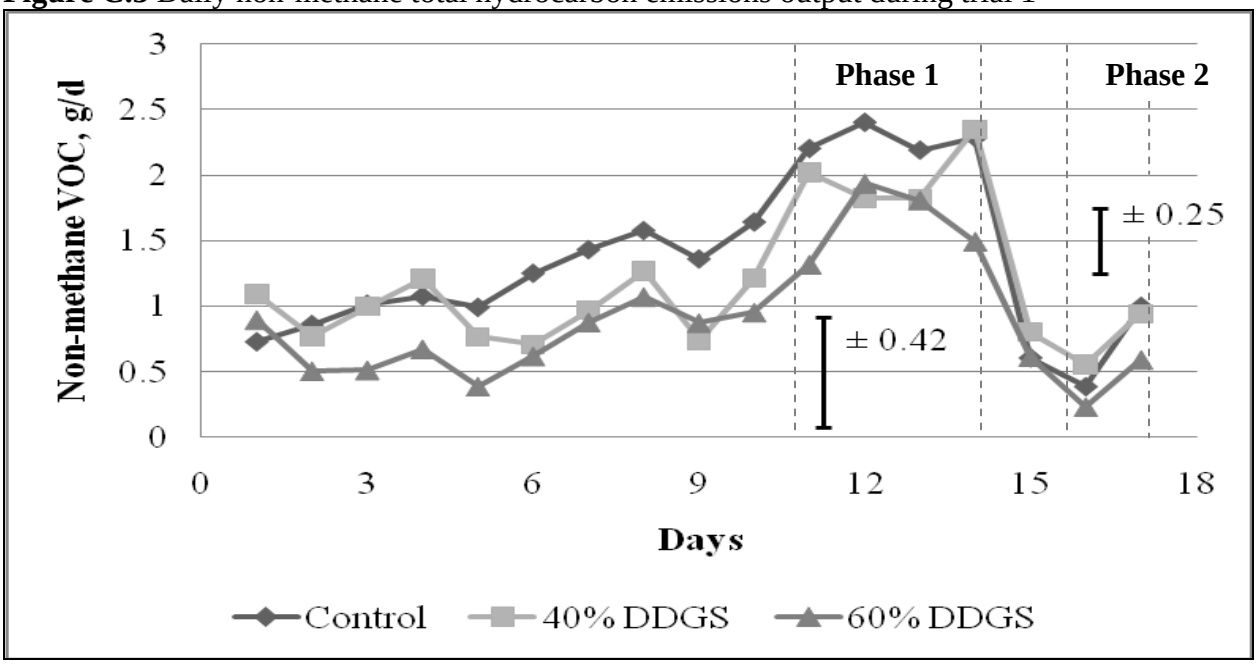
* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure C.2 Daily methane emissions adjusted for dry matter intake during trial 1



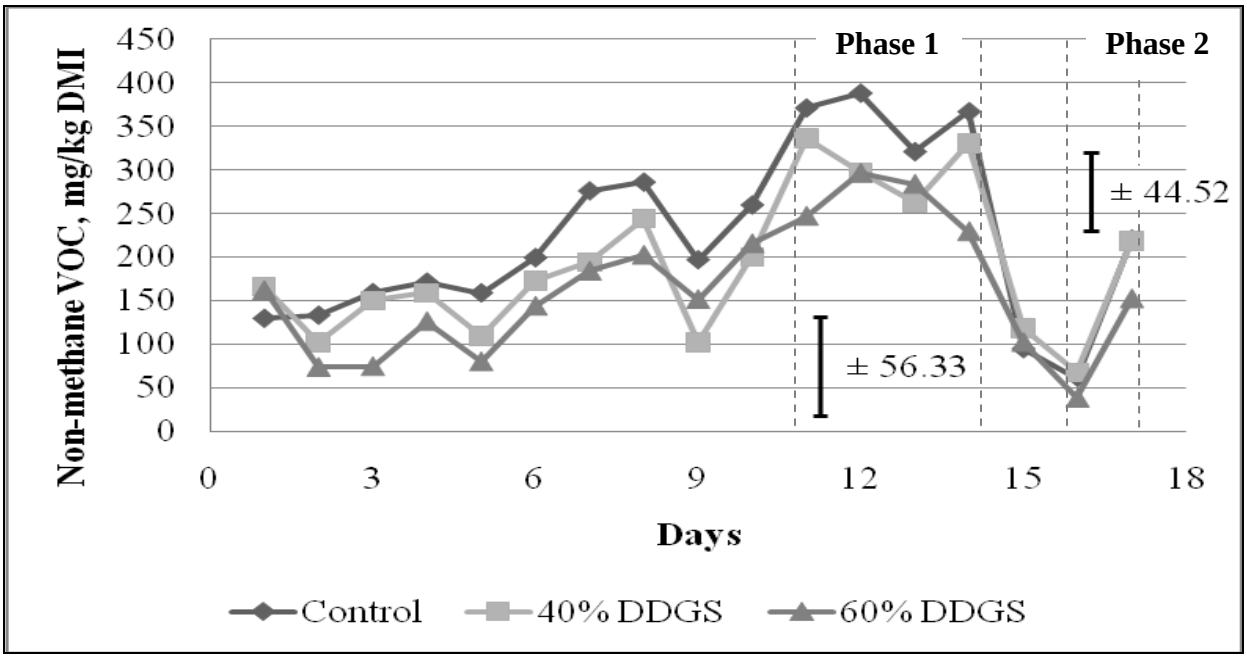
* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure C.3 Daily non-methane total hydrocarbon emissions output during trial 1



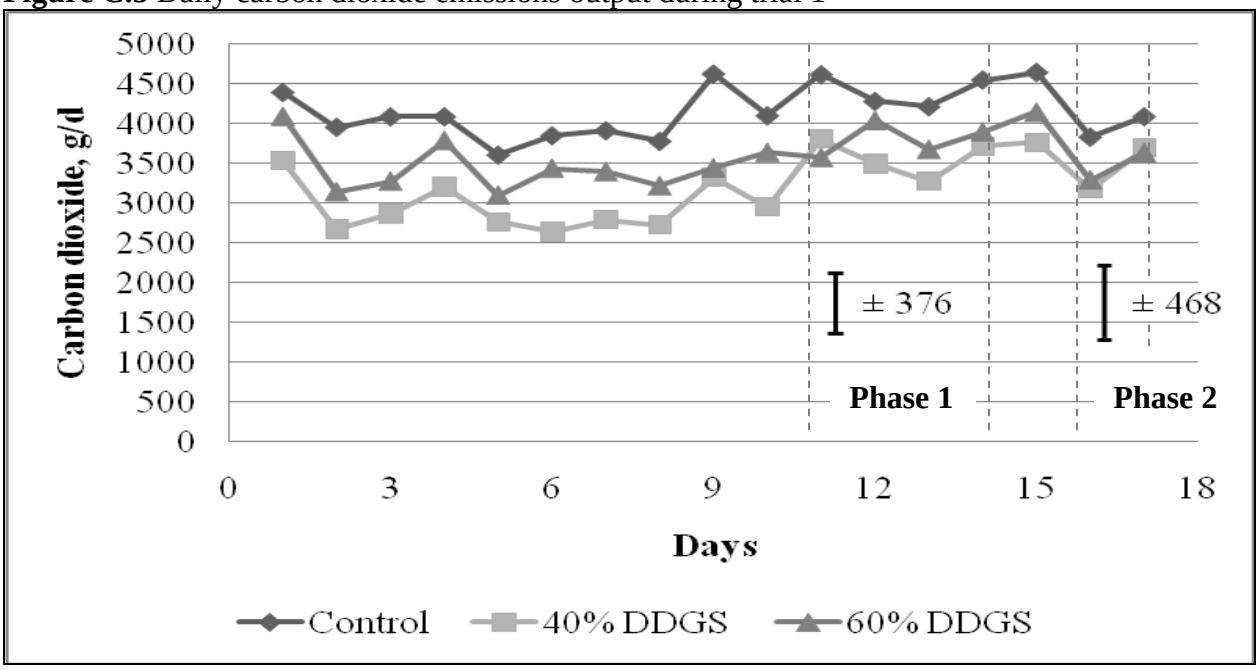
* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure C.4 Daily non-methane total hydrocarbon emissions adjusted for dry matter intake during trial 1



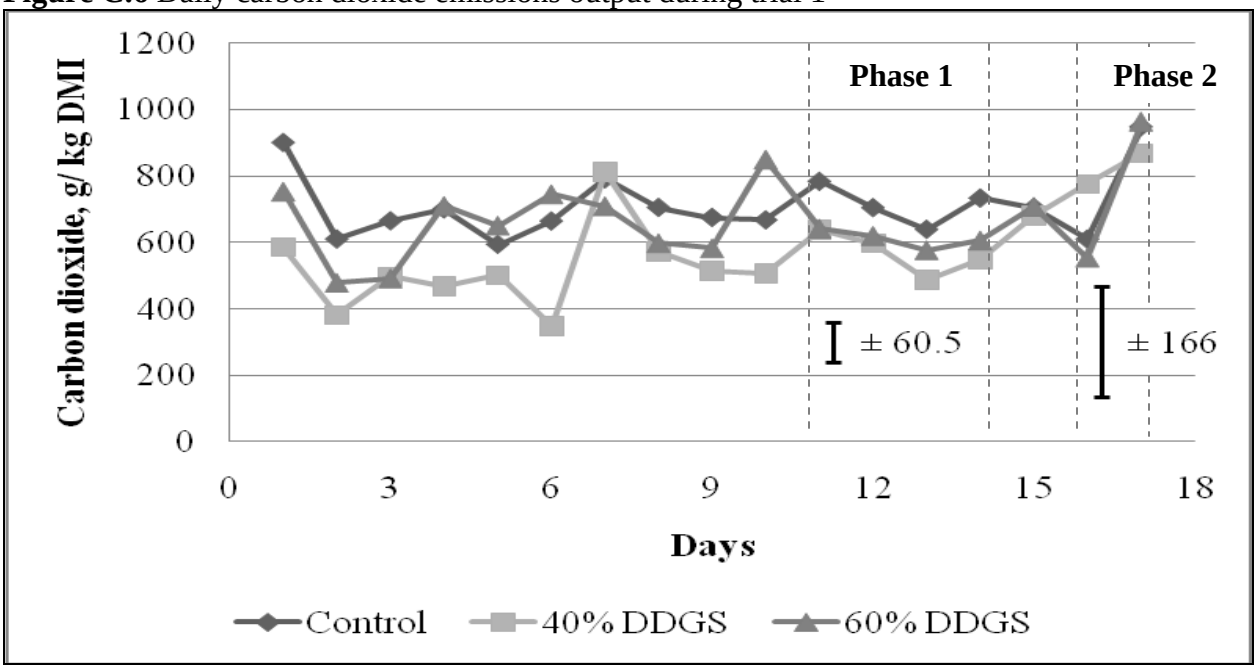
* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure C.5 Daily carbon dioxide emissions output during trial 1



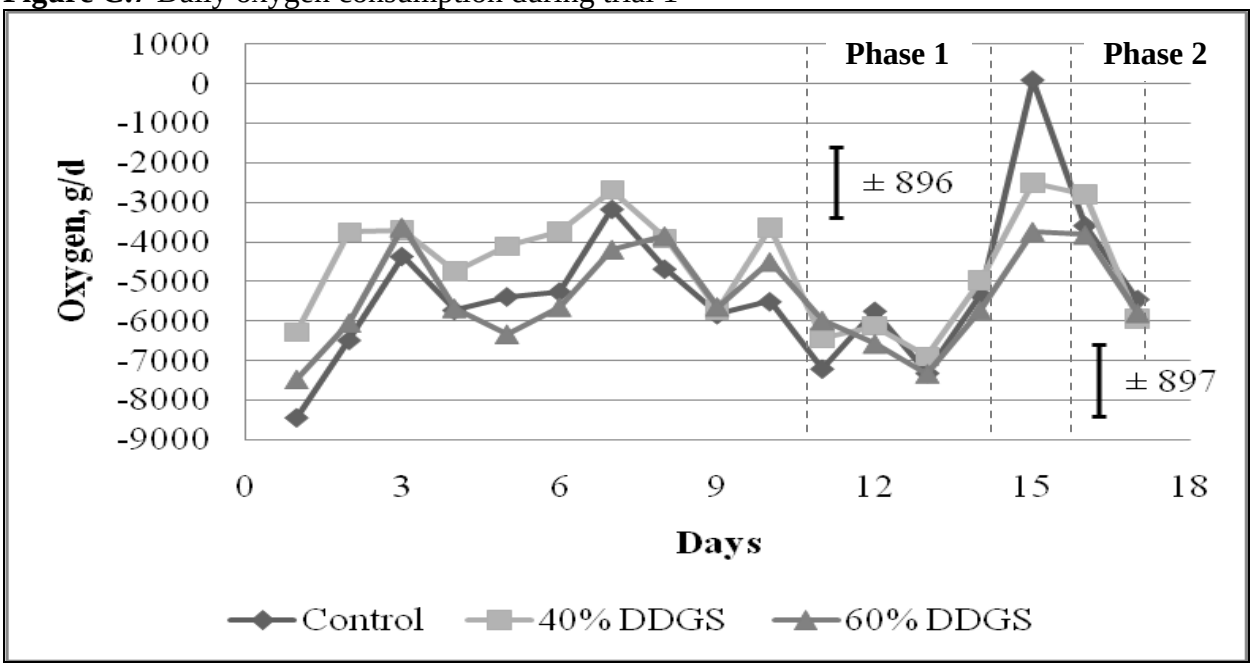
* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure C.6 Daily carbon dioxide emissions output during trial 1



* Days 11-14 represent steady state mean emissions during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure C.7 Daily oxygen consumption during trial 1



* Days 11-14 represent steady state mean consumption during phase 1. Days 16 and 17 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Table C.2 Probabilities of day effects for carbon emissions and oxygen consumption during trial 1

	Phase 1			Phase 2		
Variable	TRT	Day	TRT × Day	TRT	Day	TRT × Day
CH_4, g/d	0.38	0.99	0.84	0.44	0.11	0.70
CH_4, g/kg DMI	0.31	0.85	0.80	0.73	< 0.01	0.23
VOC, g/d	0.39	0.80	0.61	0.50	< 0.01	0.68
VOC, mg/kg DMI	0.15	0.81	0.83	0.57	< 0.01	0.73
CO_2, g/d	0.20	0.36	0.62	0.63	0.12	0.89
CO_2, g/kg DMI	0.10	0.05	0.94	0.94	0.03	0.49
O_2, g/d	0.94	0.02	0.79	0.93	< 0.01	0.14

Figure C.8 Mean methane emissions output during trial 1

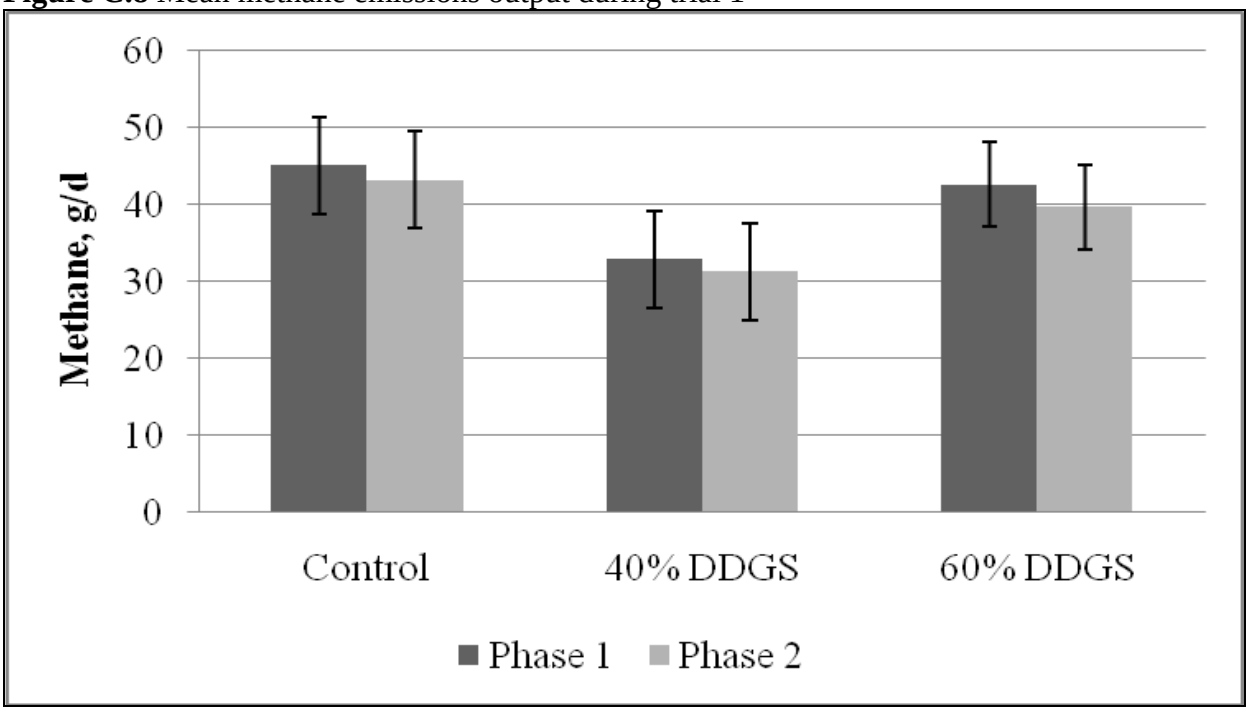


Figure C.9 Mean methane emissions adjusted for dry matter intake during trial 1

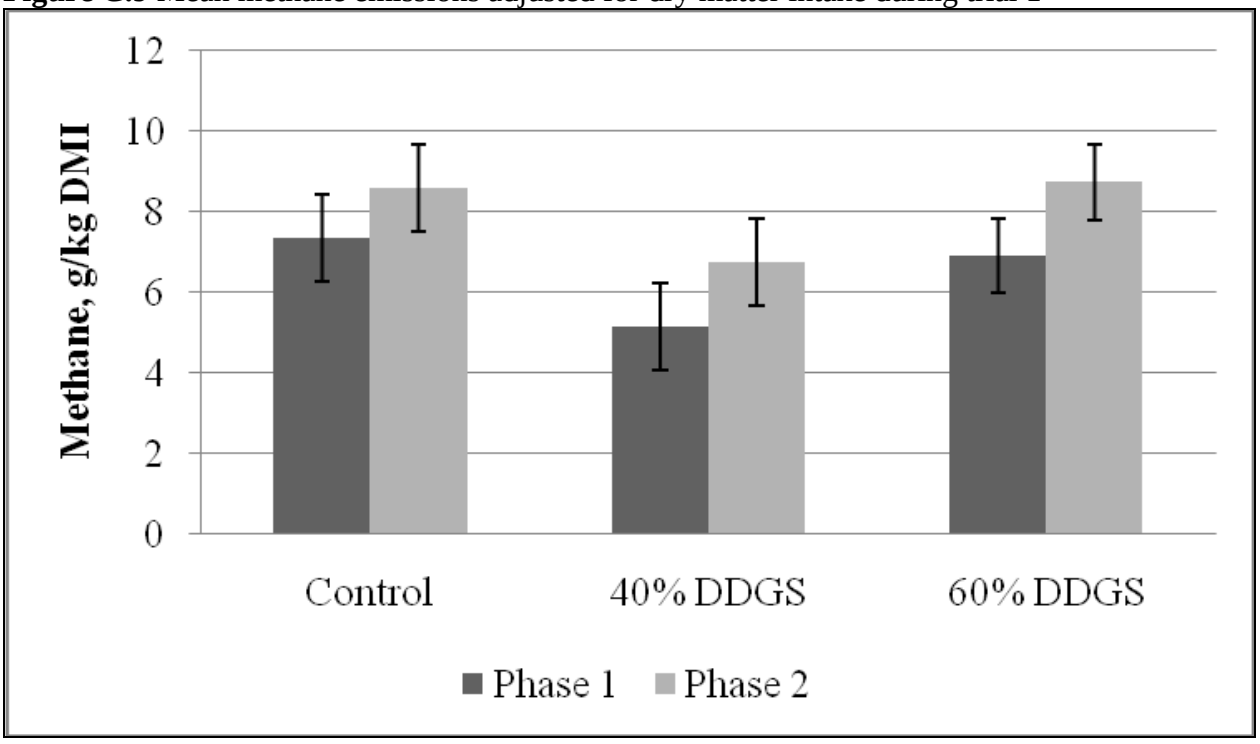


Figure C.10 Mean non-methane total hydrocarbon emissions output during trial 1

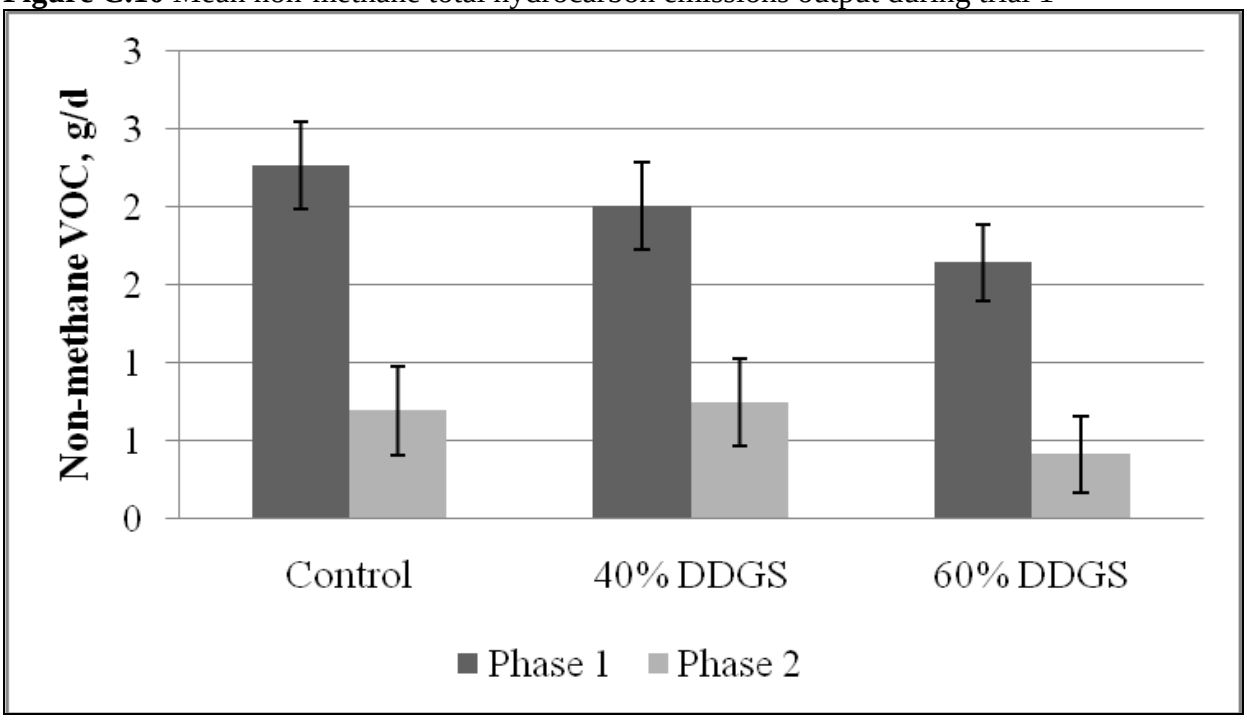


Figure C.11 Mean non-methane total hydrocarbon emissions adjusted for dry matter intake during trial 1

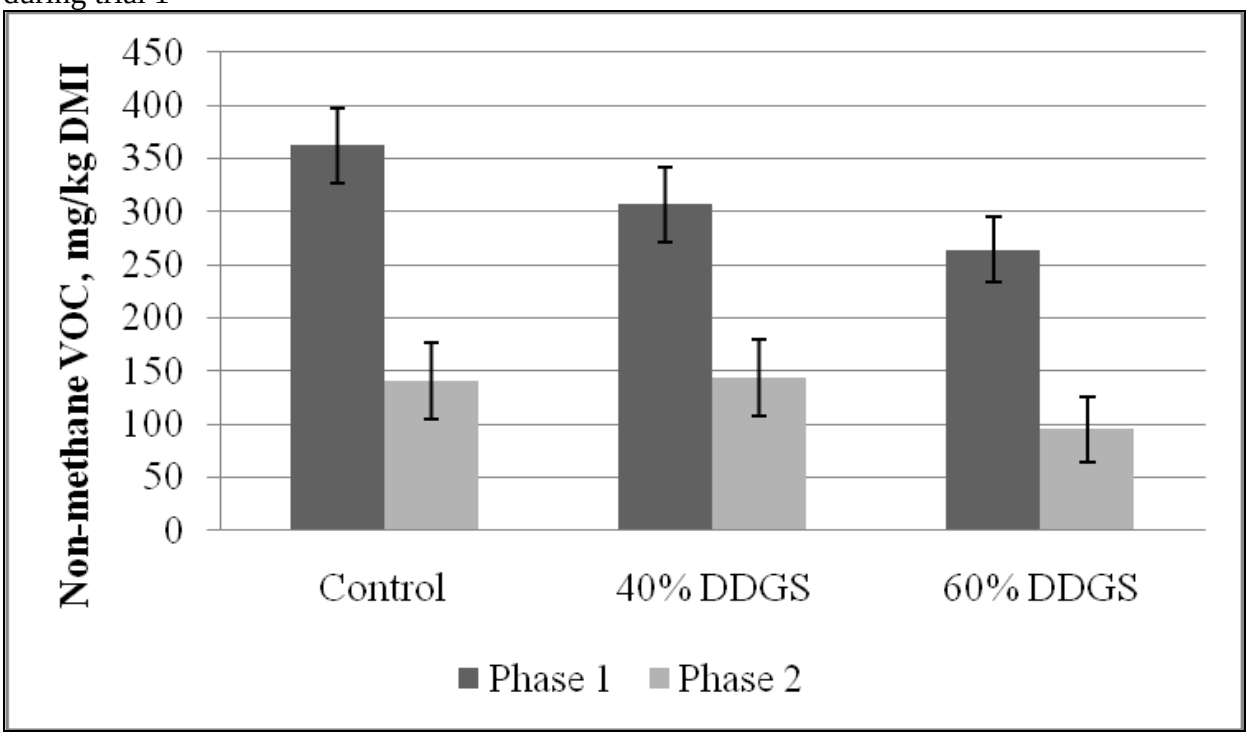


Figure C.12 Mean carbon dioxide emissions output during trial 1

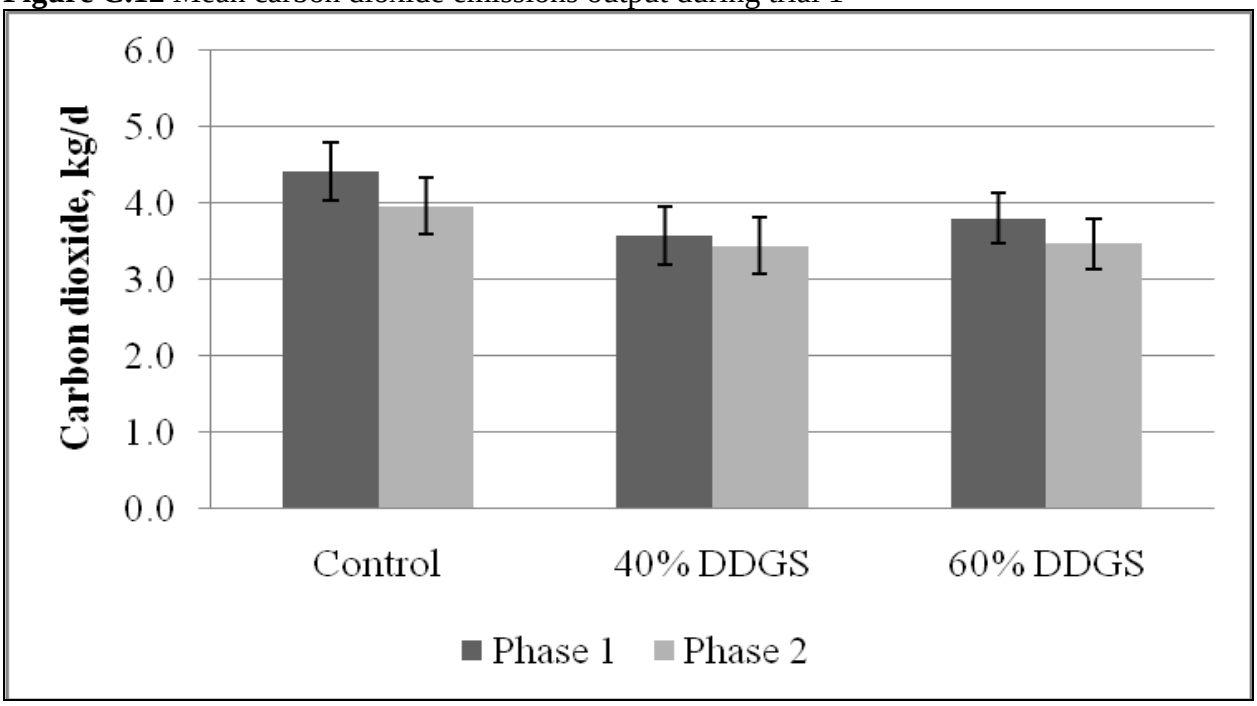
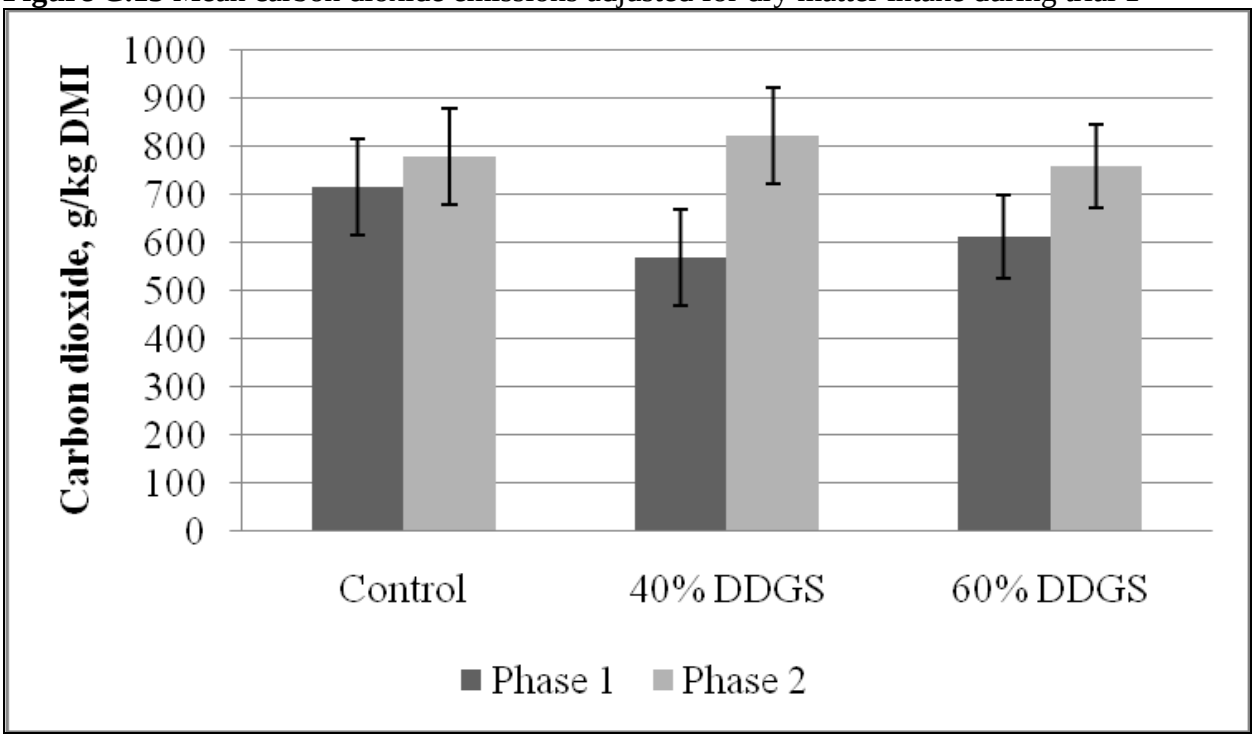


Figure C.13 Mean carbon dioxide emissions adjusted for dry matter intake during trial 1



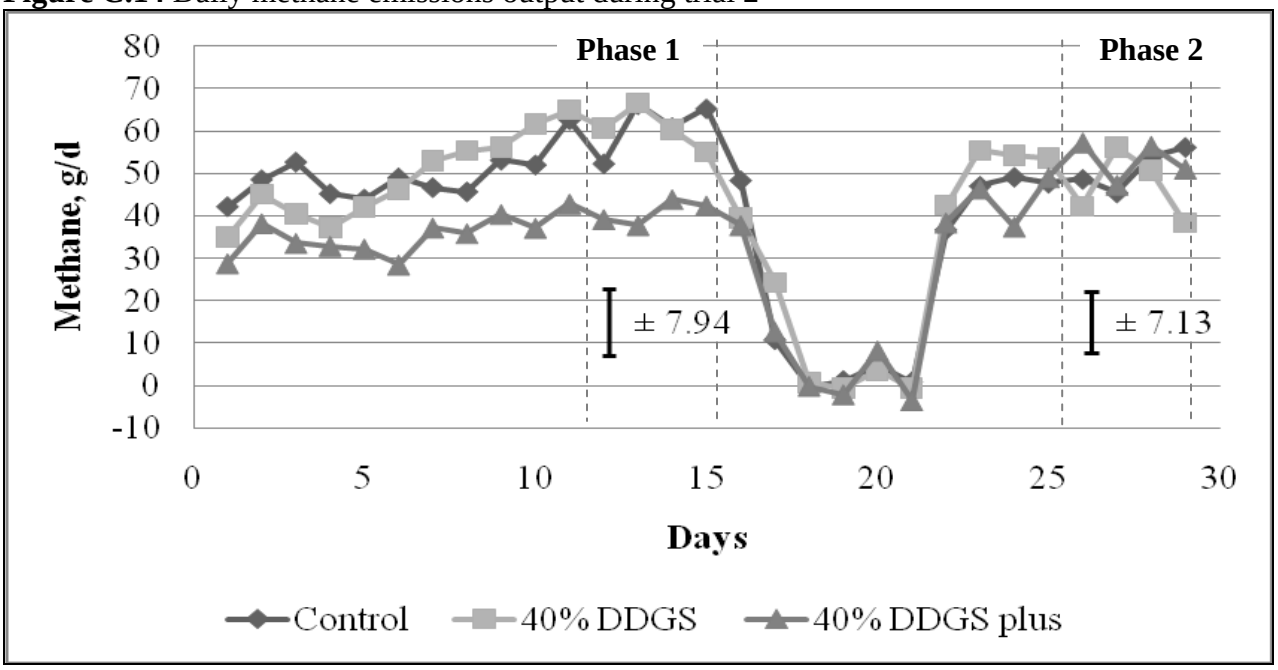
Trial 2

Table C.3 Comparison of unfiltered raw data to filtered data during trial 2

	Unfiltered raw data				
Variable	N	Mean	SD	Minimum	Maximum
Temperature,º F	28870	60.36718	1.812508	48.68827	65.35302
Humidity, %	28870	36.04142	11.30774	16.10901	104
Air flow, cfm	28870	545.1113	179.4046	0	659.829
CH_4, ppm	28870	2.134286	588.5773	-100000	9.999296
NMTHC, ppm	28870	-3.37049	588.5415	-100000	10.13834
CO_2, ppm	28870	944.197	185.3528	-7.13237	1783.55
O_2, %	28870	20.76054	0.535329	-0.02451	26.4835

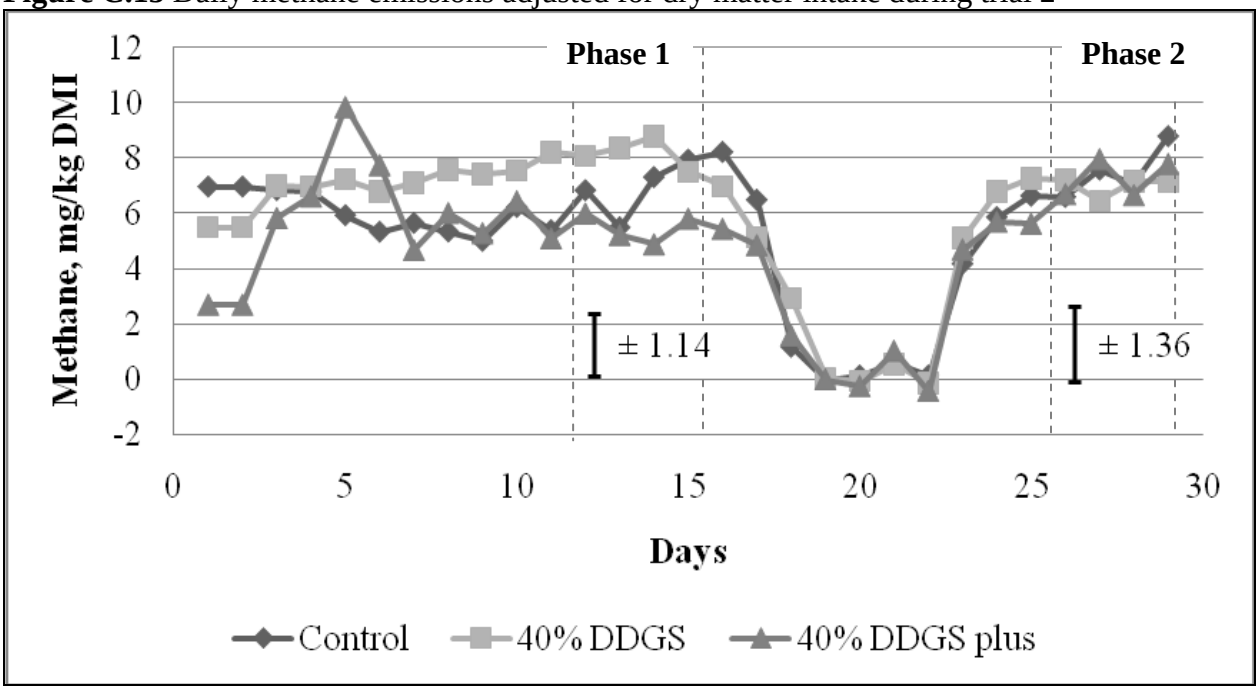
	Filtered raw data				
Variable	N	Mean	SD	Minimum	Maximum
Temperature,º F	28870	60.36718	1.812508	48.68827	65.35302
Humidity, %	28870	36.04142	11.30774	16.10901	104
Air flow, cfm	26609	591.4301	86.75048	97.00471	659.829
CH_4, ppm	28870	2.134286	588.5773	-100000	9.999296
NMTHC, ppm	28870	-3.37049	588.5415	-100000	10.13834
CO_2, ppm	28870	944.197	185.3528	-7.13237	1783.55
O_2, %	28870	20.76054	0.535329	-0.02451	26.4835

Figure C.14 Daily methane emissions output during trial 2



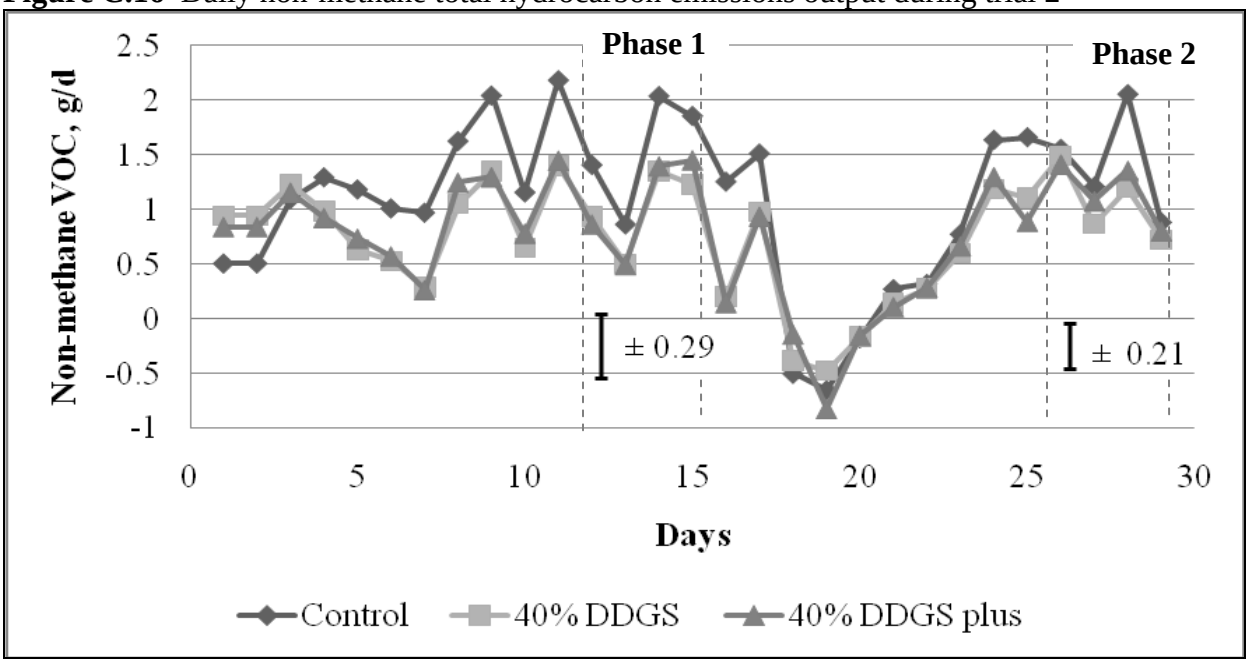
* Days 12-15 represent steady state mean emissions during phase 1. Days 26-29 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure C.15 Daily methane emissions adjusted for dry matter intake during trial 2



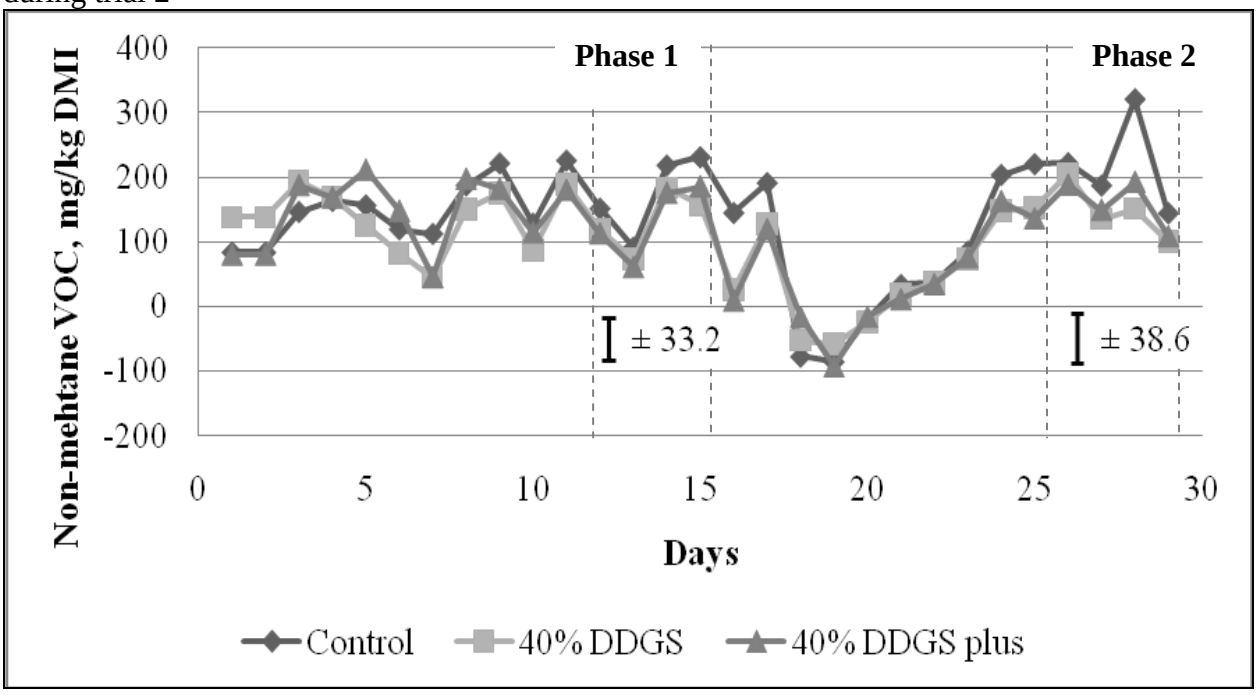
* Days 12-15 represent steady state mean emissions during phase 1. Days 26-29 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure C.16 Daily non-methane total hydrocarbon emissions output during trial 2



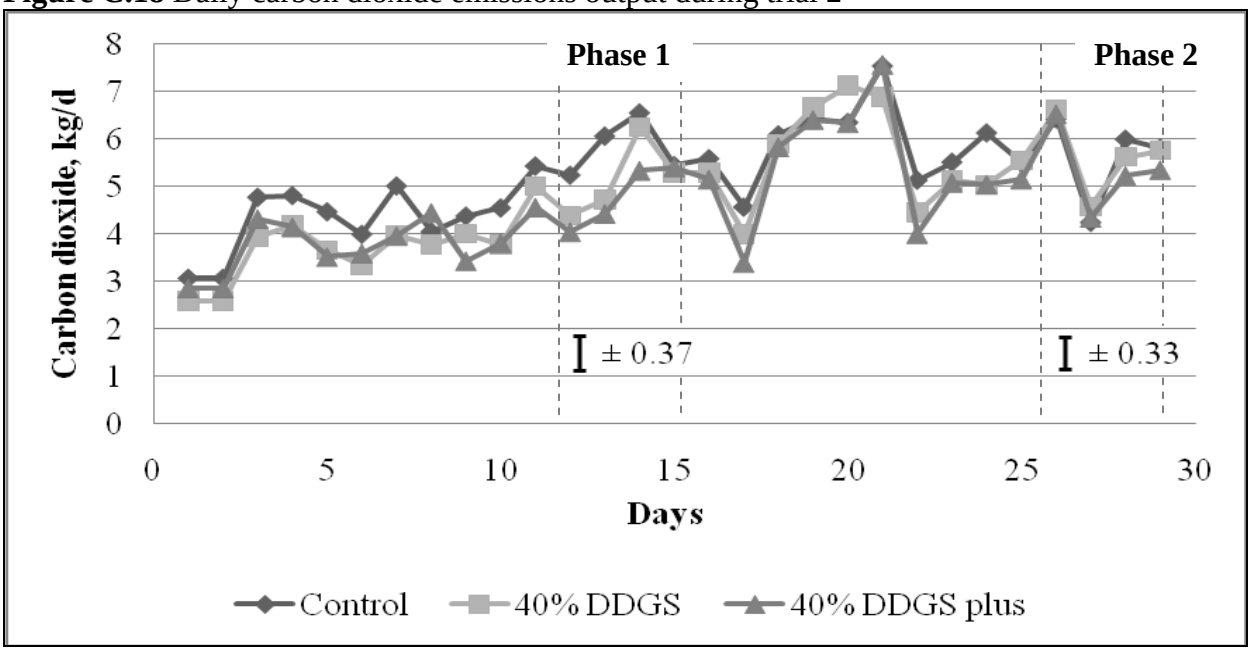
* Days 12-15 represent steady state mean emissions during phase 1. Days 26-29 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure C.17 Daily non-methane total hydrocarbon emissions adjusted for dry matter intake during trial 2



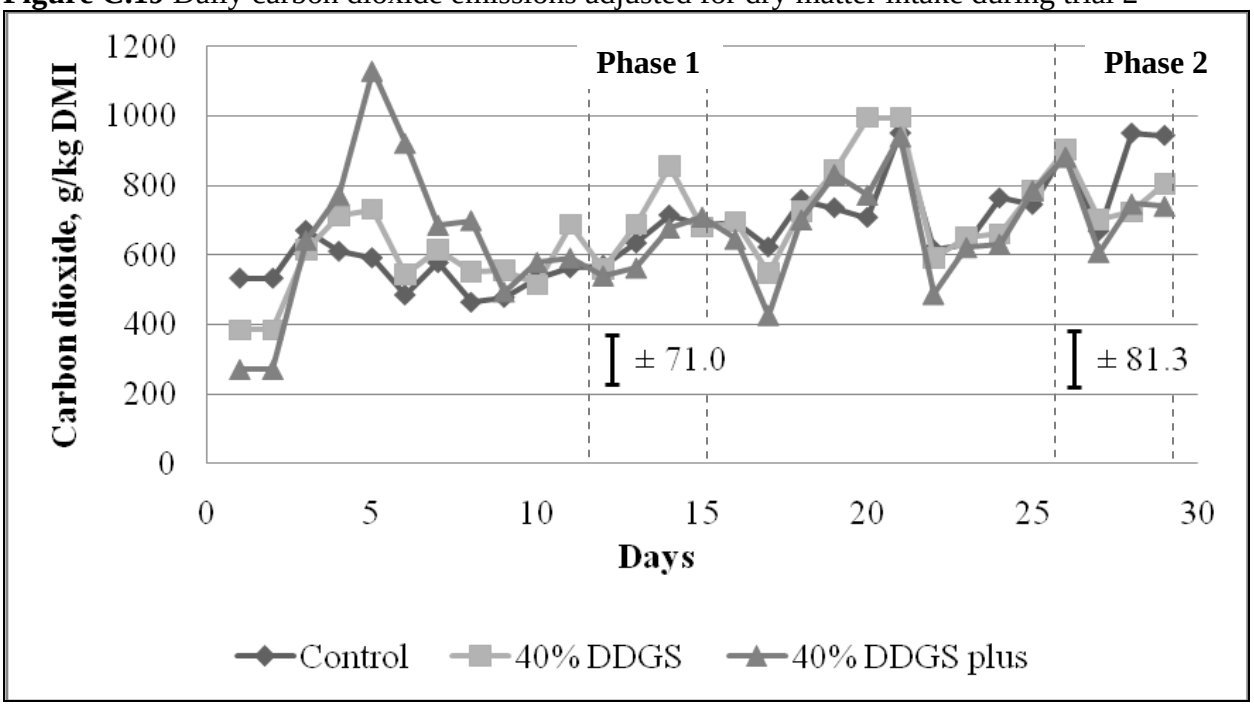
* Days 12-15 represent steady state mean emissions during phase 1. Days 26-29 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure C.18 Daily carbon dioxide emissions output during trial 2



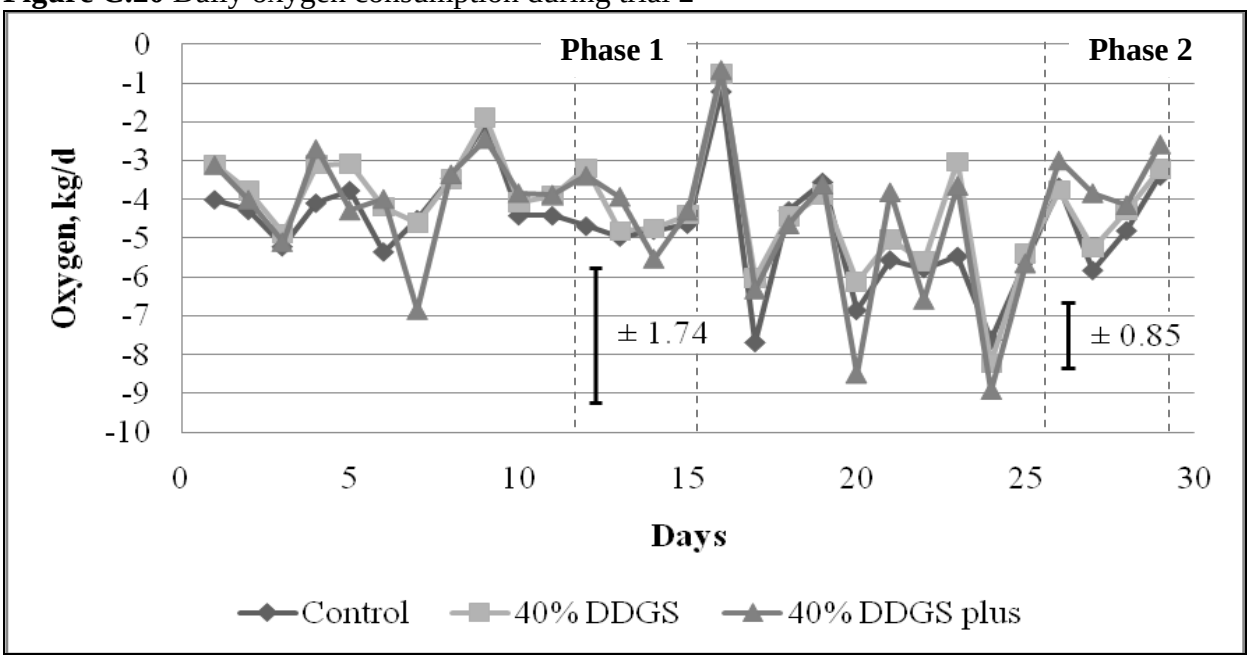
* Days 12-15 represent steady state mean emissions during phase 1. Days 26-29 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure C.19 Daily carbon dioxide emissions adjusted for dry matter intake during trial 2



* Days 12-15 represent steady state mean emissions during phase 1. Days 26-29 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Figure C.20 Daily oxygen consumption during trial 2



* Days 12-15 represent steady state mean consumption during phase 1. Days 26-29 represent means for phase 2. Error bars for phase 1 and phase 2 represent the SEM

Table C.4 Probability of day effects for carbon emissions and oxygen consumption during trial 2

	Phase 1			Phase 2		
Variable	TRT	Day	TRT × Day	TRT	Day	TRT × Day
CH_4, g/d	0.12	0.32	0.20	0.78	0.37	0.01
CH_4, g/kg DMI	0.21	0.59	0.08	0.45	< 0.01	0.42
VOC, g/d	0.08	< 0.01	0.72	< 0.01	< 0.01	0.24
VOC, mg/kg DMI	0.06	< 0.01	0.58	< 0.01	0.02	0.09
CO_2, g/d	0.03	0.02	0.30	0.55	< 0.01	0.74
CO_2, g/kg DMI	0.61	0.05	0.47	0.25	< 0.01	0.62
O_2, g/d	0.66	0.04	0.58	0.23	< 0.01	0.77

Figure C.21 Mean methane emissions output during trial 2

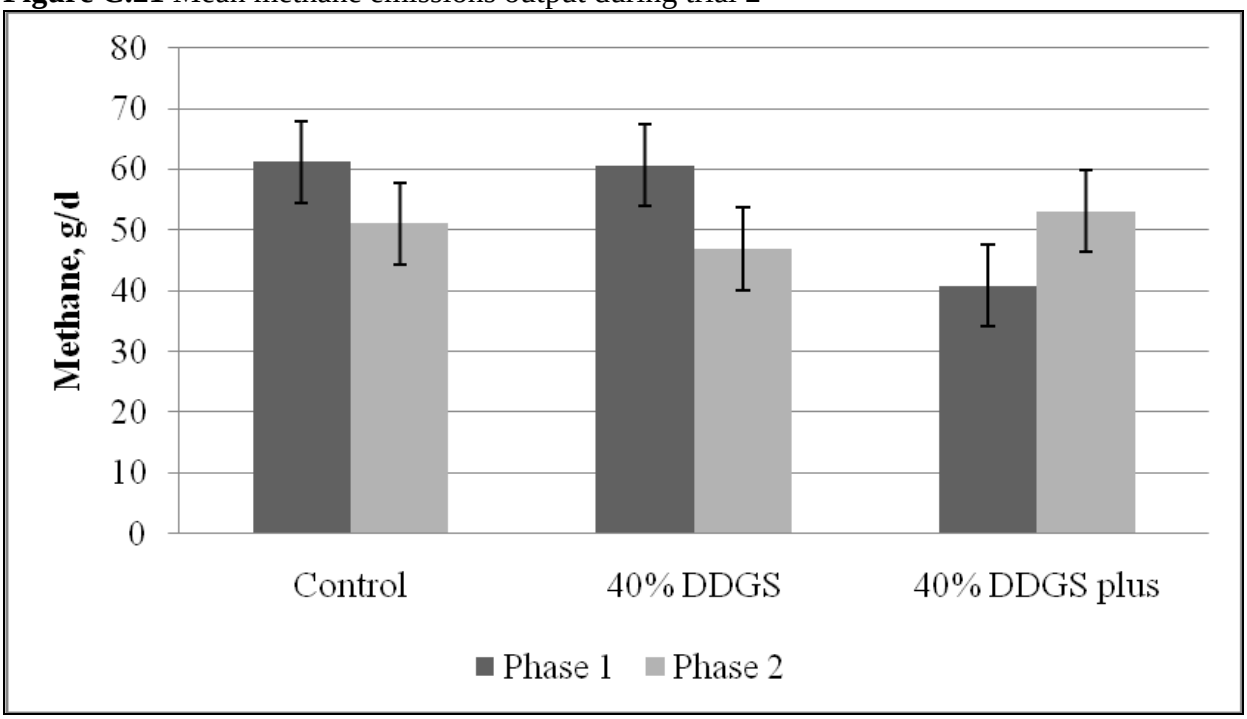


Figure C.22 Mean methane emissions adjusted for dry matter intake during trial 2

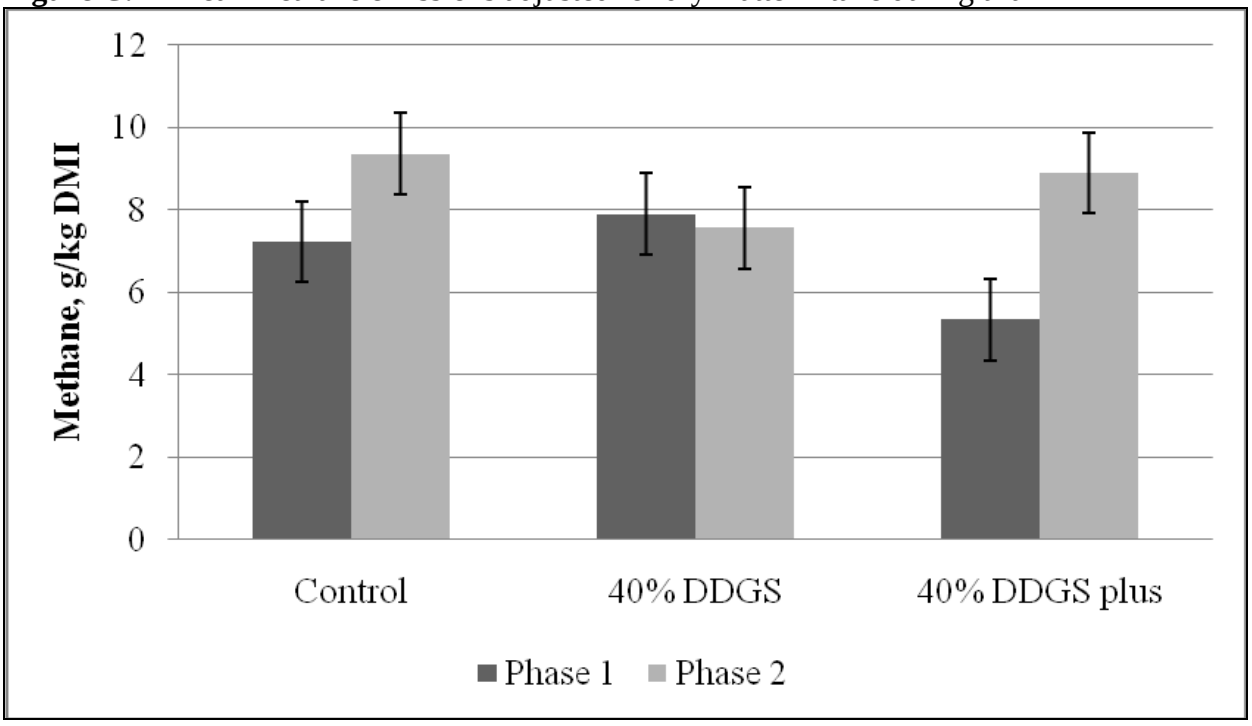


Figure C.23 Mean non-methane total hydrocarbon emissions output during trial 2

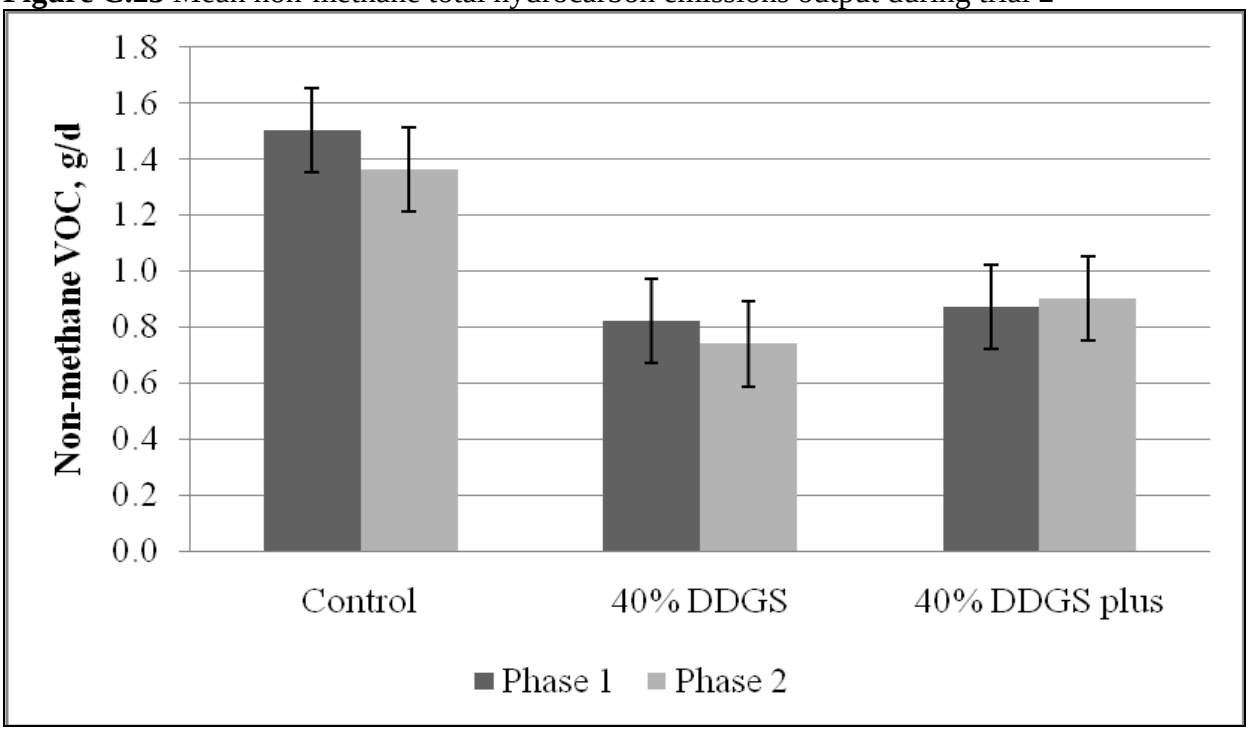


Figure C.24 Mean non-methane total hydrocarbon emissions adjusted for dry matter intake during trial 2

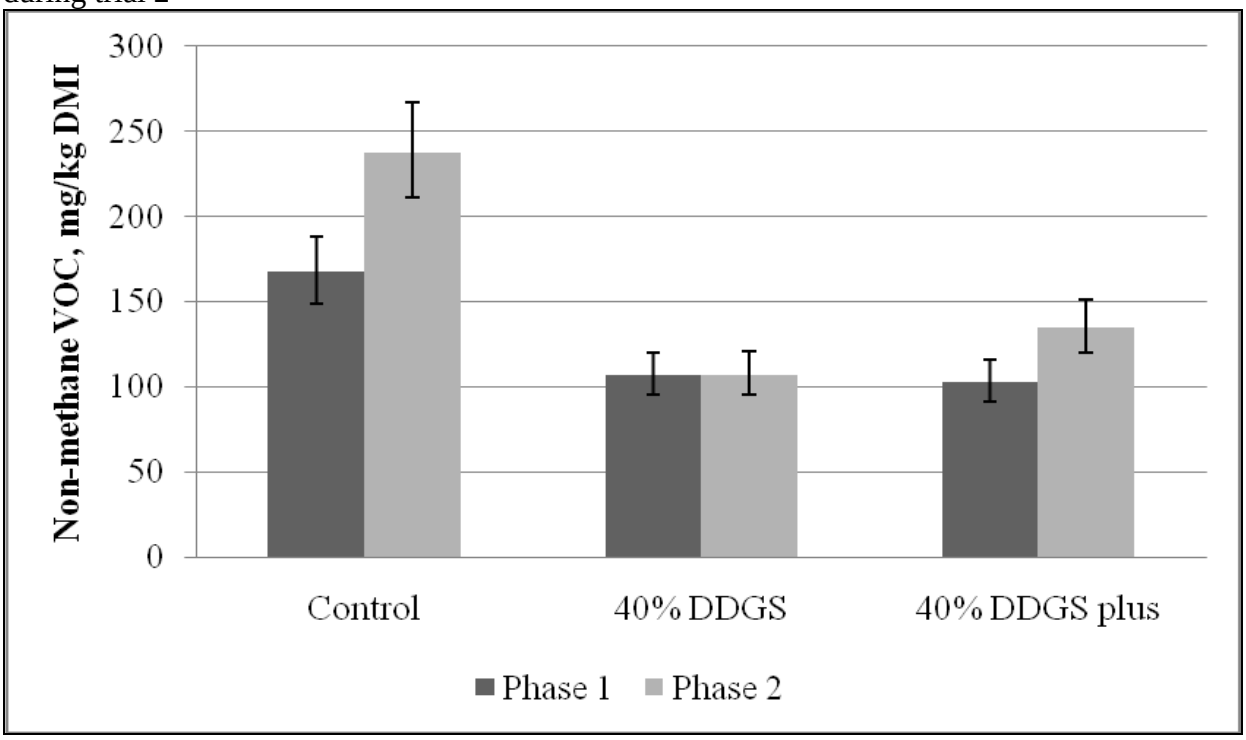


Figure C.25 Mean carbon dioxide emissions output during trial 2

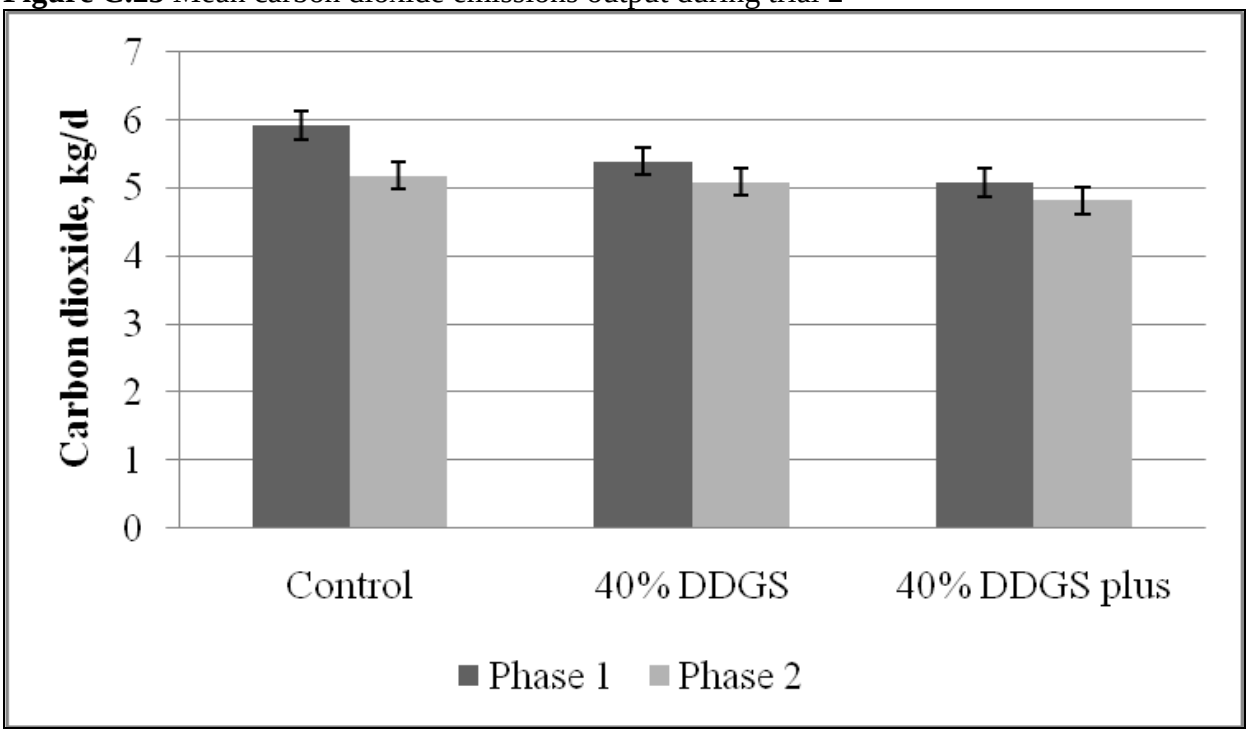
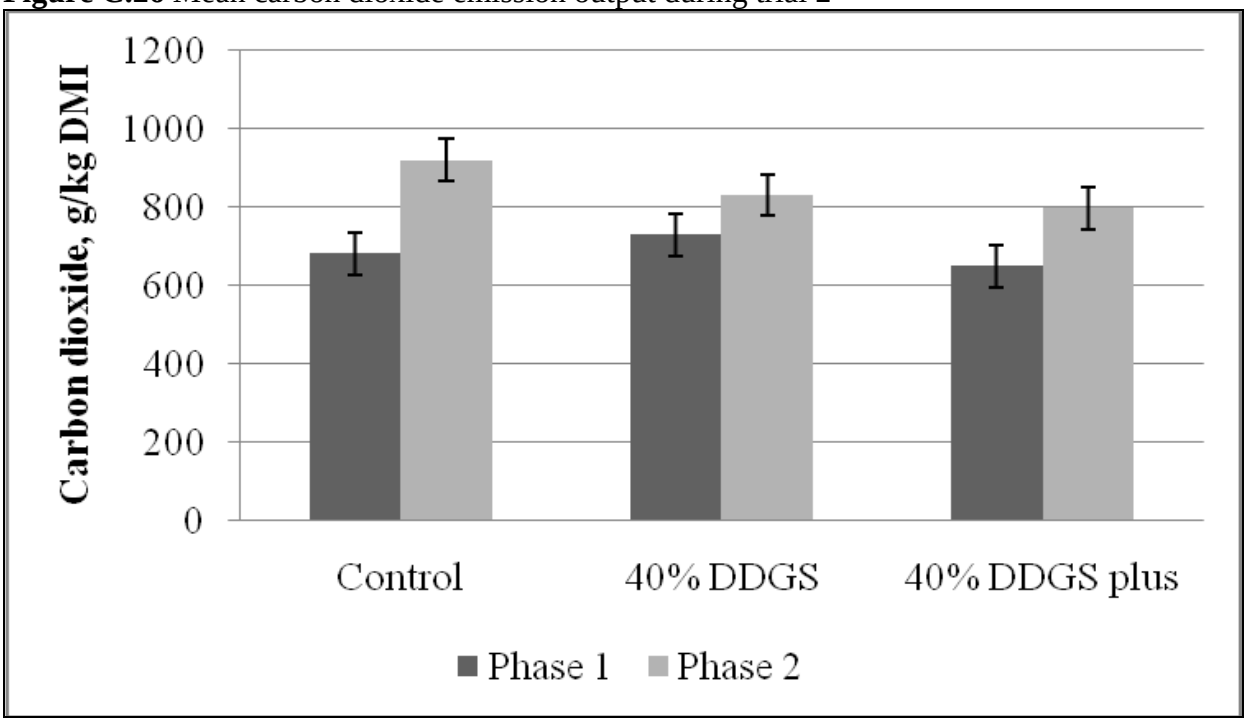


Figure C.26 Mean carbon dioxide emission output during trial 2



APPENDIX D: DETERMINATION OF COPPER AND MOLYBDENUM LEVELS IN FERMENTATION VESSELS TO MITIGATE SULFIDE GAS PRODUCTION.

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SUMMARY

Laboratory experiments were conducted to determine the effects of different or ranging levels of Mo and Cu in high concentrate diets as a strategy to mitigate H_2S emissions. Two experiments were conducted; experiment 1 used 125 ml fermentation vessels with 10 ml of rumen fluid, 10 ml of Van Soest media without reducing agent, 0.5 g of DDGS as a substrate, along with various combinations of Mo and Cu in solution added to each vessel. Concentrations included; 0, 3, 6, and 9 ppm Mo in combination with 0, 30, 60, and 90 ppm Cu using 2 Cu sources, $CuCl_2$ and $CuSO_4$. In experiment 2, Mo and Cu concentrations increased 10-fold, using 0, 30, 60, and 90 ppm Mo in combination with 0, 300, 600, and 900 ppm Cu. Vessels for each experiment were tested in duplicate and incubated at 39° C for 16 h. Results from experiment 1 demonstrated a quadratic effect for H_2S production from Cu treatments only ($P < 0.01$). In experiment 2, both Mo and Cu linearly decreased H_2S emissions as treatment levels increased ($P < 0.01$). In both experiments, lower emitted levels of H_2S gas were observed when $CuCl_2$ was added ($P < 0.01$) compared to $CuSO_4$. Treatment with Mo and Cu did not affect microbial fermentation based on overall gas production, IVDMD, and pH.

Key words: Copper, molybdenum, distiller's grain with soluble, in vitro, hydrogen sulfide

INTRODUCTION

Distiller's grain with soluble (**DGS**) is a co-product from ethanol production that has become a popular feed source to livestock, particularly feedlot cattle. During production of DGS, sulfuric acid is added during the fermentation process to regulate pH and as a cleaning agent, and this increases the amount and concentration of S in DGS (Vannes et al., 2009a; Kelzer et al., 2010). High levels of sulfates and other forms of dietary S are reduced in the rumen by sulfate-reducing bacteria (**SRB**) to produce hydrogen sulfide (**H_2S**) and other ionic forms that are not only hazardous to the animal but can cause potential air quality issues by increased H_2S emissions. Excess generation of H_2S in the rumen will depress normal rumen function and could lead to respiratory problems (Kandylis, 1983), as the majority of belched gas is inhaled into the lungs (Bulgin et al., 1996). Hydrogen sulfide gas is dangerous for humans to inhale (Osweiler et al., 1985; Gerber et al., 1991). Excess H_2S in ambient air can have a detrimental impact to the environment, such as acid rain, and eutrophication (Guang-hui et al., 2007).

Molybdate (**MoO_4**) inhibits SRB by working as an analog of sulfate, blocking the sulfate activation step that is catalyzed by ATP sulfurylase (Oremland and Capone, 1988). Sodium molybdate decreases H_2S production in cattle fed high S diets, although results were not consistent among cattle (Loneragan et al., 1998). Additionally, the study from Loneragan et al. (1998) showed that inclusion of MoO_4 dramatically decreased liver Cu stores in cattle. Molybdenum (**Mo**) and S react to form tetrathiomolybdates that react with copper (**Cu**) and particulate matter in the rumen. This results in the formation of highly stable compounds that cannot be digested and absorbed (Allen and Gawthorne, 1987; Suttle, 1991). The synergetic

effect between Mo and S begins with the substitution of S for oxygen in the MoO_4^{2-} ion to ultimately yield tetrathiomolybdate (MoS_4^{2-}).

$$MoO_4^{2-} \rightarrow MoO_3S^{2-} \rightarrow MoO_2S_2^{2-} \rightarrow MoOS_3^{2-} \rightarrow MoS_4^{2-}$$

Tetrathiomolybdate has the potential to bind ruminal Cu ions, rendering the entire complex biologically unavailable to the animal.

The objective of these experiments was to determine if various combinations of Cu and Mo would decrease H_2S gas productions within an in vitro system. In experiment 1, concentrations were chosen based on maximum tolerable concentrations set by the NRC (2000). Concentrations selected were 0, 30, 60, and 90 ppm Cu from 2 different Cu sources and 0, 3, 6, 9 ppm Mo. In the 2nd experiment, the concentrations selected were greater than experiment 1 so we could determine if 10-fold greater concentrations would alter sulfide gas production as well as on microbial viability. A report from Huber et al. (1971) indicated feeding Mo up to 100 ppm over 6 months showed no signs of toxicity. However, inclusion levels as high as 100 ppm may not be necessary in order to mitigate sulfide gas production as more recent research has shown sulfide gas production to be decreased by greater than 70% using 25 ppm Mo of the liquid within fermentation vessels (Kung et al., 2000).

MATERIALS AND METHODS

Experiment 1

The experiment was conducted using in vitro batch fermentations to determine the appropriate concentrations of Mo and Cu to potentially reduce H_2S production. Approval for this study was provided by the Michigan State University Animal Care and Use Committee (AUF # 07/09-110-00). A 4 × 4 × 2 factorial arrangement of treatments were used as our experimental design with 4 levels of Mo (0, 3, 6, and 9 ppm) and 4 levels of Cu (0, 30, 60, and 90 ppm) and 2 Cu sources. Sodium molybdate (**Na_2MoO_4**) was the compound used as the Mo source and the 2 Cu sources were copper chloride (**$CuCl_2$**) and copper sulfate (**$CuSO_4$**). Potential solubility differences between the Cu sources may impact the formation of the indigestible triad compound. Calculations for dilutions are shown in Tables D.4-D.6. The amount of Cu and Mo added to each fermentation vessel were calculated based on the amount of substrate (0.5 g). Serum bottles (125 ml) served as fermentation vessels to which the dried distiller's grain with soluble (**DDGS**) substrate (Table D.1), 10 ml Van Soest buffer media without the reducing agent (Table D.1), 10 ml of strained rumen fluid, and 0.2 ml of various mineral treatments were added. Two fermentation vessels containing buffer and rumen fluid served as negative controls.

Rumen fluid collected for in-vitro fermentation procedures was obtained from a ruminally cannulated cow housed at Michigan State University Dairy Teaching and Research Center (**DTRC**). The cow was fed a diet containing 50% alfalfa hay, 30% DDGS, 16.5% dry ground corn, and 3.5% vitamin and mineral pre-mix. The cow was adjusted to the DDGS concentrate diet and was allotted a minimum of 28 d to acclimate before rumen fluid was collected. Rumen fluid was collected taking 2 samples near the rumen-reticulo opening, 1 within

the ventral sac, and 2 near the posterior blind-sac to provide a representative sample of SRB population throughout the rumen (Lyford and Church, 1988).

Table D.1 Composition of distiller's grain with soluble, and the Van Soest buffer media

DDGS compostition	% DM
Dry matter	87.0
Crude protein	27.0
Crude fat	7.5
Crude fiber	13.0
Sulfur	0.7

Van Soest media*	ml/vessel
Distilled water	4.99
Trypticase® peptone[1]	0.02
Micromineral solution[2]	0.001
Rumen buffer solution[3]	2.49
Macromineral solution[4]	2.49
Resazurin	0.001
Total	9.99

[1] Trypticase® peptone used was a pancreatic digest of casein (Becton Dickinson BBL #4311921

[2] Micromineral solution contained 13.2 g $CaCl_2 \times 2H_2O$, 10.0 g $MnCl_2 \times 4H_2O$, 1.0 g $CoCl_2 \times 6H_2O$, and 8.0 g $FeCl_3 \times 6H_2O$ to 100 ml of distilled water

[3] Rumen buffer solution contained 4.0 g ammonium bicarbonate and 35 g of sodium bicarbonate to 1 L of distilled water

[4] Macromineral solution contained 5.7 g Na_2HPO_4 anhydrous, 6.2 g KH_2PO_4 anhydrous, and 0.6 g $MgSO_4 \times 7H_2O$ to 1 L of distilled water

* Van Soest media was prepared in the ingredient order listed from top to bottom

Bottles were capped with rubber stoppers and crimped to create a closed system and placed in an orbital shaker incubator (Forma Scientific) at 39° C for 16 h. Bottles were covered to allow in vitro fermentation to occur in the dark to best simulate conditions during ruminal fermentation. After 16 h, bottles were removed from the incubator at approximately 1 m 45 s intervals in the same order they were set in the incubator to allow homogenous incubation time between all serum bottles. Sixteen h incubation time was selected to represent approximate retention time in the rumen (Phillip et al., 1980). An in vitro study conducted by Kahlon et al. (1975) suggested 6 to 18 h incubation time was required for SRB to synthesize various S-containing sources into microbial protein. Once the fermentation vessel was removed from the incubator, a syringe needle attached to a digital pressure gauge (MediaGauge^{TM}, SSI Technologies, Inc.) was inserted through the rubber stopper and pressure (psi) was measured and recorded. Pressure (psi) and known head space was then used to calculate total gas volume (ml) using Boyle's Gas Law at STP, which states:

$$G_p = V_h/P_t \times P_a$$

or

$$V_h = G_p/P_a \times P_t$$

where G_p is the total gas volume of the head space (ml), P_a is the atmospheric pressure (psi) and P_t is the reading from the digital pressure gauge (Mauricio et al., 1999). The pressure gauge needle was then removed and a second needle connected to a 100 cc glass syringe (B-D YALE, Becton, Dickerson & Co., U.S.A.) was inserted to obtain a sample of the gas. The gas pressure within the fermentation vessel forced gas into the glass syringe until the system reached equilibrium pressure between the serum bottle and syringe. When the system was at equilibrium,

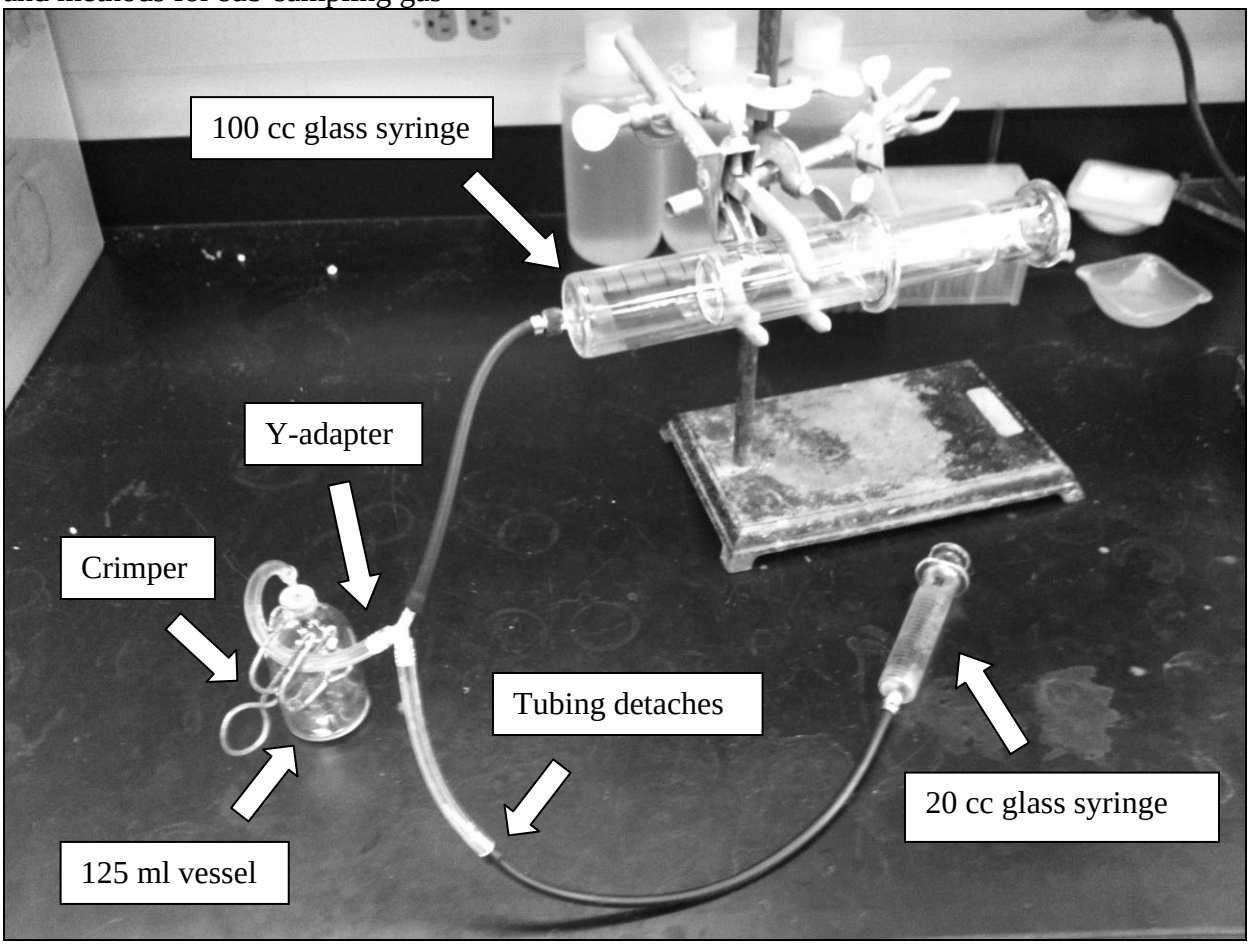
the tubing connecting the needle to the syringe was clamped to trap the gas in the glass syringe. A 10 ml sub-sample was then extracted from the 100 cc glass syringe using a 20 ml glass syringe (B-D YALE, Becton, Dickerson & Co., U.S.A.) that was attached to the tubing using a Y-adapter. Tubing was crimped accordingly to prevent gas loss and direct the gas into the 20 ml glass syringe (Figure D.1). Gas from each fermentation vessel collected in the syringe was then bubbled in 10 ml of pH 10.00 water in 50 ml glass test tubes to trap S-containing gas in a liquid state (Seigel, 1965; Kung, 1998). Gas was bubbled in a more alkaline solution using a pH of 10 compared to procedures described by Kung (1998) to reduce potential gas loss of sulfur. Below pH 10, H_2S can volatilize and escape from the solution as a gas (Cord-Ruwisch, 1985). This process was repeated for all fermentation vessels.

Sulfide determination was performed using the methylene blue (**MB**) reaction; adding 1 ml of 0.02 M of *N, N*-dimethyl-*p*-phenylene diamine sulfate (**DPD**) in 7.2 N HCl and 1 ml 0.03 M ferric chloride in 1.2 N HCl to each test tube. The DPD reagent is oxidized by Fe^{3+}, which reacts with H_2S to form MB (Monares et al., 2010).

All test tubes were vortexed and placed in the dark for approximately 20 to 30 minutes to allow complete color formation. A standard was prepared from sodium sulfide ($Na_2S{\cdot}9H_2O$) using methods described by others (Siegel, 1965; Kung, 1998; Vanness et al., 2009b). Sulfur in solution (µmol /ml) was determined by pipeting 200 µl of the standard solution in plate reader wells and comparing absorbencies at a wavelength of 630 nm to the standard curve on a spectrophotometer (Figure D.2). Plates were arranged by copper treatments; all $CuCl_2$ with Mo

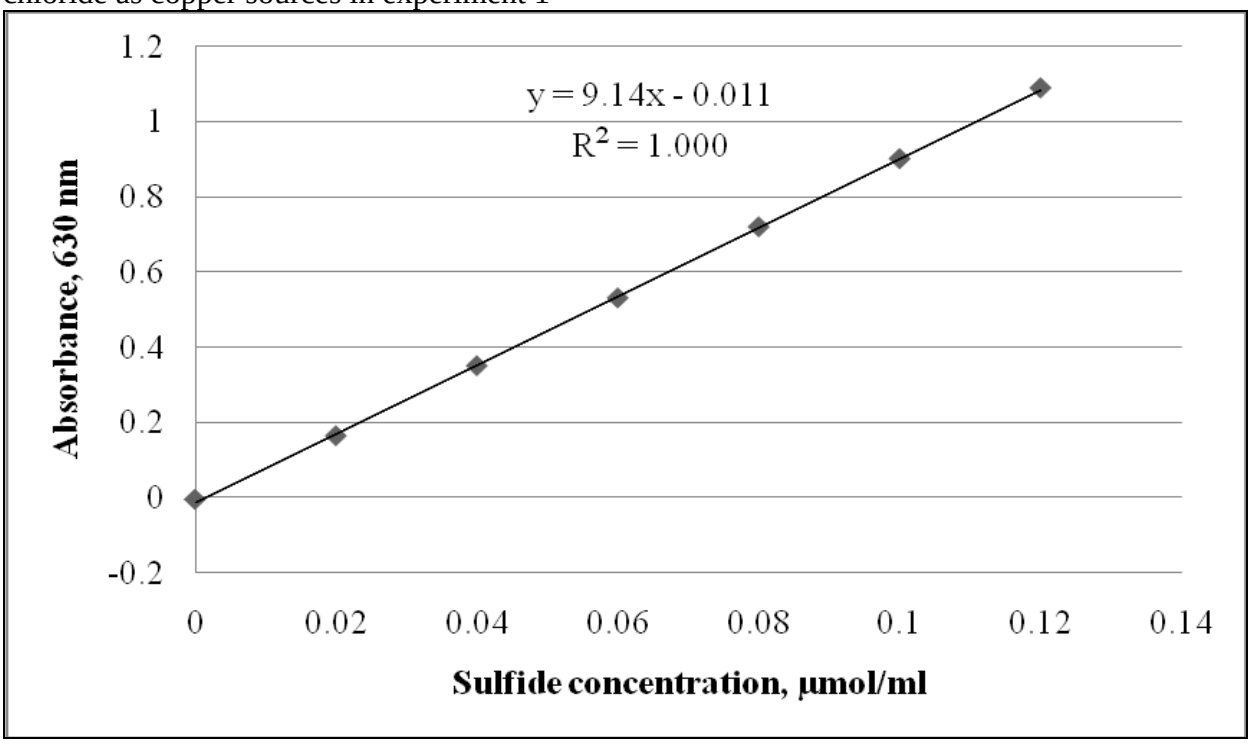
combinations were on a single plate, and all $CuSO_4$ with Mo combinations were pipetted onto a different plate.

Figure D.1 Image illustrating the removal of gas from the head space of the fermentation vessels and methods for sub-sampling gas



* The rubber tubing for the 20 cc glass syringe was crimped before tubing was detached to bubble sub-sample into alkaline water

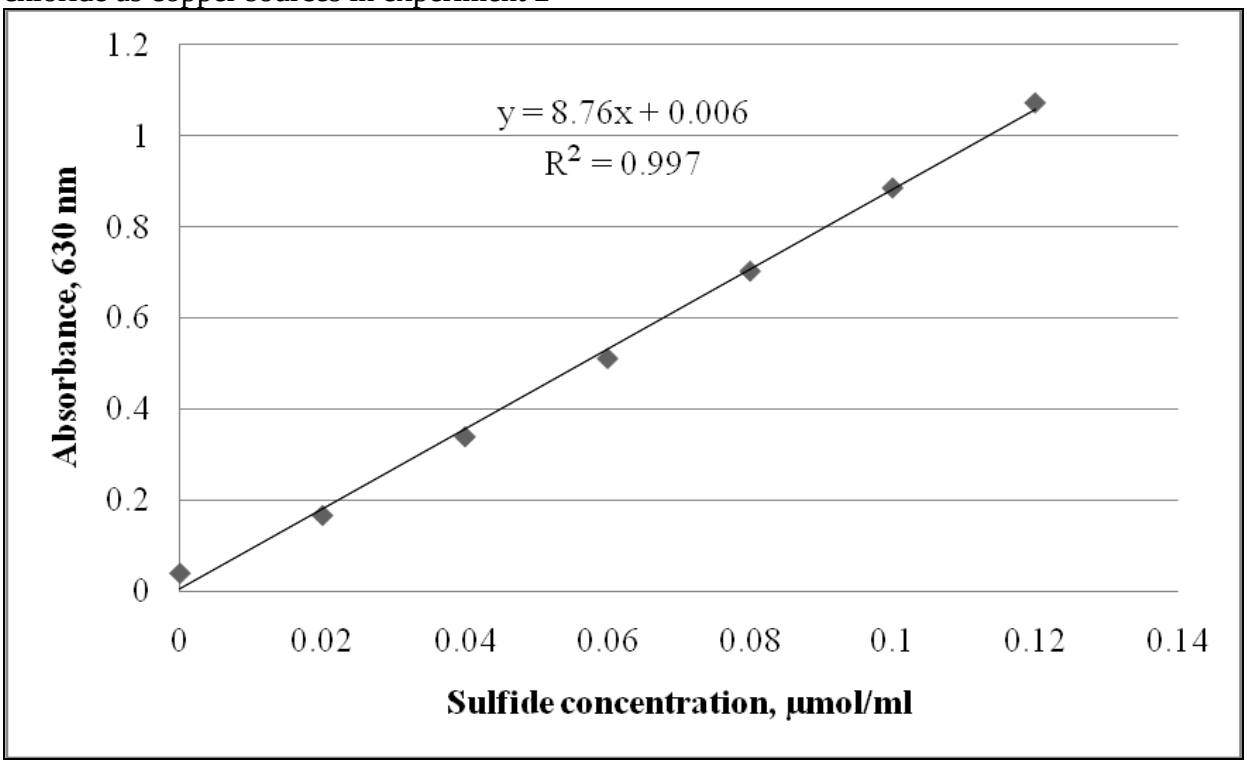
Figure D.2 Standard curve for all treatment combinations using both copper sulfate and copper chloride as copper sources in experiment 1



Experiment 2

A second experiment was conducted using a 10-fold greater Mo and Cu concentrations than in experiment 1. A similar factorial arrangement of treatments was used as described in experiment 1. The concentrations of Cu and Mo were (0, 300, 600, and 900 ppm) and (0, 30, 60, 90 ppm), respectively. Procedures were similar to those described in experiment 1, except for the following changes. Gas was bubbled into pH 8 water as opposed to using pH 10 water in experminent 1. Also, the entire gas extracted from each serum bottle was bubbled in the water compared to only 10 ml being used in experiment 1. Testing within our laboratory determined similar results from using either method. Hydrogen sulfide concentrations were calculated using a similar procedure as experiment 1 by comparing the absorbency to a calibration curve (Figure D.3).

Figure D.3 Standard curve for all treatment combinations using both copper sulfate and copper chloride as copper sources in experiment 2



Statistical analysis

Statistical analysis was performed using PROC MIXED sub-routine of SAS 9.2 (SAS Inst., Inc., Cary, NC). Data was analyzed as a randomized complete block design with a 4 × 4 × 2 factorial arrangement of treatments. Analysis was run using concentrations of Mo, Cu, and Cu source as the independent variables for experiments 1 and 2. The dependent variables reported were molar units of H_2S produced, H_2S produced per g of degraded substrate **DM**, in-vitro dry matter disappearance (**IVDMD**), and pH. Main effects of Mo and Cu level were analyzed to determine differences across treatment levels. The molar unit of gas produced in the fermentation vessels were corrected for the molar unit of gas produced in the blanks (vessels containing no DDGS substrate). Interaction effects between Cu level, Mo level, and Cu source are provided in Table D.8. Each experiment was analyzed separately. All dependent variables were tested for homogeneous variance using Bartlett's test and for normal distribution using Shapiro Wilk's test. Statistical significance was declared at a *P*-value at or below 0.05 and trends at *P*-value at or below 0.10.

RESULTS

Experiment 1

2 or 3-way interactions between Cu level, Cu source, and Mo level for H_2S production were not significant (Table D.8). Copper levels at 30 and 60 ppm from both $CuSO_4$ and $CuCl_2$ had similar nmols of H_2S produced, percent of IVDMD, and pH. However, the decrease in nmols of H_2S produced ($P < 0.01$), H_2S per g of degraded substrate ($P = 0.01$), and pH ($P < 0.01$) was determined when comparing 90 ppm with 30 and 60 ppm Cu from the $CuCl_2$ source only (Table D.2). Treatment of Cu demonstrated a quadratic effect in the nmols of H_2S produced and H_2S adjusted for DM degradation across Cu levels ($P < 0.01$) for both Cu sources. However, CuSO4 also showed a linear increase ($P < 0.01$) as Cu treatments increased H_2S production compared to the 0 ppm Cu vessels.

The pH using $CuCl_2$ ($P < 0.01$) linearly decreased and a quadratic effect was identified using $CuSO_4$ ($P = 0.04$; Table D.2).The pH of the fermented solution was more acidic at 9 ppm Mo compared to 6 ppm Mo ($P = 0.04$), but similar to the 0 and 3 ppm Mo treatments. The pH of Mo showed a quadratic effect ($P = 0.04$), as the 3 and 6 ppm Mo treatments had a greater pH compared to the 0 and 9 ppm Mo treatments. Additionally, the percent of IVDMD tended to show a quadratic effect for Mo levels ($P = 0.05$) and Cu levels from $CuSO_4$ ($P < 0.01$).

There was a tendency for a 3-way interaction between Cu level, Cu source, and Mo level when considering the percent of IVDMD ($P = 0.06$; Table D.8). Only IVDMD ($P = 0.09$) and pH ($P = 0.06$) showed a tendency for a 2-way interaction between Mo level and Cu source. Finally,

only pH expressed a tendency ($P = 0.04$) for a 2-way interaction between Mo level and Cu level. However, the statistical differences in pH between copper sources are unlikely of any biological significance as the pH of all Cu levels between each Cu source is within a ± 0.05 pH range. No significant differences were expressed for the 2-way interaction between Cu level and Cu source; however, Cu source as a main affect did differ between nmols of H_2S, H_2S adjusted on DM degradation, and pH ($P < 0.01$). Copper sources also tended to cause different IVDMD ($P = 0.06$).

Table D.2 Effects of molybdenum and copper levels on the fermentation of rumen fluid using distiller's grain with soluble as a substrate during experiment 1

	Molybdenum, ppm						Contrast	
Item	0	3	6	9	SEM	*P*-value	Linear	Quadratic
H_2S, nmol	735.2	759.2	759.0	759.3	27.7	0.84	0.48	0.88
H_2S, µmol/g[1]	3.18	3.18	3.10	3.25	0.13	0.88	0.82	0.57
IVDMD, %	44.73	46.03	46.51	45.37	0.68	0.29	0.43	0.08
pH	5.69[ab]	5.70[ab]	5.70[a]	5.68[b]	0.004	0.01	0.07	0.04

	Copper from $CuCl_2$, ppm							
Item	0	30	60	90	SEM	*P*-value	Linear	Quadratic
H_2S, nmol***	555.8[a]	712.4[b]	698.5[b]	594.9[a]	33.9	< 0.01	0.48	< 0.01
H_2S, µmol/g***	2.53[a]	3.17[b]	3.13[b]	2.58[a]	0.17	0.01	0.88	< 0.01
IVDMD, %*	43.89	45.22	44.95	46.21	0.90	0.35	0.11	0.97
pH***	5.70[a]	5.69[a]	5.69[a]	5.66[b]	0.005	< 0.01	< 0.01	0.17
	Copper from $CuSO_4$, ppm							
H_2S, nmol	555.8[a]	838.2[b]	854.6[b]	820.4[b]	33.9	< 0.01	< 0.01	< 0.01
H_2S, µmol/g	2.53[a]	3.54[b]	3.65[b]	3.64[b]	0.17	< 0.01	< 0.01	< 0.01
IVDMD, %	43.89[a]	47.48[b]	46.70[b]	45.17[b]	0.90	0.04	.046	< 0.01
pH	5.70	5.71	5.70	5.69	0.005	0.08	0.16	0.04

[1] Represents µmol of H_2S per g of degraded DDGS on a DM basis

[a, b] Means without common superscripts within a row differ

*** Copper source differ ($P \leq 0.01$); *Copper source tend to differ ($P \leq 0.10$)

Experiment 2

Greater treatment differences were demonstrated when Mo and Cu treatments were increased 10-fold compared to experiment 1 (Table D.3). Treatment of Mo and Cu from both $CuCl_2$ and $CuSO_4$ showed a significant linear reduction in the µmols of H_2S produced and H_2S emissions adjusted for DM degradation as the treatment inclusion level increased from 0, 300, 600, to 900 ppm ($P < 0.01$). A quadratic effect was also shown in the µmols of H_2S produced ($P = 0.04$) and H_2S emissions adjusted for DM degradation ($P < 0.01$) when $CuCl_2$ was used as the Cu source (Table D.3). Copper sulfate also demonstrated a tendency for a quadratic effect ($P = 0.09$) for µmols of H_2S produced. When comparing treatment differences for $CuCl_2$, 300 ppm differed from 600 and 900 ppm for the µmols of H_2S produced ($P < 0.05$). For $CuSO_4$, each level of Cu treatment differed in µmols of H_2S produced ($P < 0.01$) with 900 ppm Cu emitting the lowest levels of H_2S.

Hydrogen sulfide production was mitigated by increasing Cu and Mo inclusion levels. Furthermore, it is interesting that the treatment of Mo and Cu at these levels did not seem to affect the percent of IVDMD and pH; suggesting Cu and Mo concentration up to 900 and 90 ppm, respectively, does not alter in-vitro microbial fermentation. Copper sulfate expressed linear increase in pH ($P < 0.01$) as Cu levels increased. The 300 ppm Cu treatment differed ($P = 0.01$) from the 0, 600, and 900 ppm Cu levels from $CuSO_4$. Copper treatment from $CuCl_2$ increased pH compared to vessels without Cu treatment ($P < 0.01$; Table D.3). Despite statistical differences in pH, it is unlikely that pH had any biological effect on microbial viability as the pH levels for all treatments were within typical pH range (5.5-7.2) for ruminal micro-organisms

(Kung, 2008). Copper chloride treatment had a lower reduction in µmols of H_2S produced ($P <$ 0.01), while producing a greater average pH ($P < 0.01$) compared to Cu treatments from $CuSO_4$. Additionally, a significant 2-way interaction was present with Cu level by Mo level for µmols of H_2S produced ($P = 0.02$) as reported in Table D.8. The 2-way interaction for µmols of H_2S produced is further illustrated in Figure D.5.

Table D.3 Effects of molybdenum and copper levels on the fermentation of rumen fluid using distiller's grain with soluble as a substrate during experiment 2

	Molybdenum, ppm						Contrast	
Item	0	30	60	90	SEM	*P*-value	Linear	Quadratic
H_2S, µmol††	2.39[a]	2.09[ab]	2.13[ab]	1.73[b]	0.09	< 0.01	< 0.01	0.58
H_2S, µmol/g[1, ††]	11.10[a]	9.41[b]	9.75[ab]	7.86[c]	0.42	< 0.01	< 0.01	0.81
IVDMD, %	43.2	43.36	43.25	43.98	0.57	0.72	0.47	0.86
pH	5.88	5.87	5.92	5.90	0.03	0.56	0.40	0.74

	Copper from $CuCl_2$, ppm							
Item	0	300	600	900	SEM	*P*-value	Linear	Quadratic
H_2S, µmol***	4.03[a]	2.26[b]	1.30[c]	0.99[c]	0.13	< 0.01	< 0.01	0.04
H_2S, µmol/g***	18.21[a]	10.45[b]	5.90[c]	4.46[c]	0.57	< 0.01	< 0.01	< 0.01
IVDMD, %	43.99	43.08	44.11	44.07	0.78	0.48	0.69	0.55
pH***	5.86[a]	6.06[b]	6.08[b]	6.04[b]	0.04	< 0.01	< 0.01	< 0.01
	Copper from $CuSO_4$, ppm							
H_2S, µmol	4..27[a]	2.93[b]	1.86[c]	1.19[d]	0.13	< 0.01	< 0.01	0.09
H_2S, µmol/g	18.52[a]	13.34[b]	8.79[c]	5.53[d]	0.57	< 0.01	< 0.01	0.15
IVDMD, %	45.76	43.85	42.68	43.02	0.78	0.75	0.20	0.75
pH	5.86[a]	5.70[b]	5.75[ab]	5.75[ab]	0.04	0.01	< 0.01	0.08

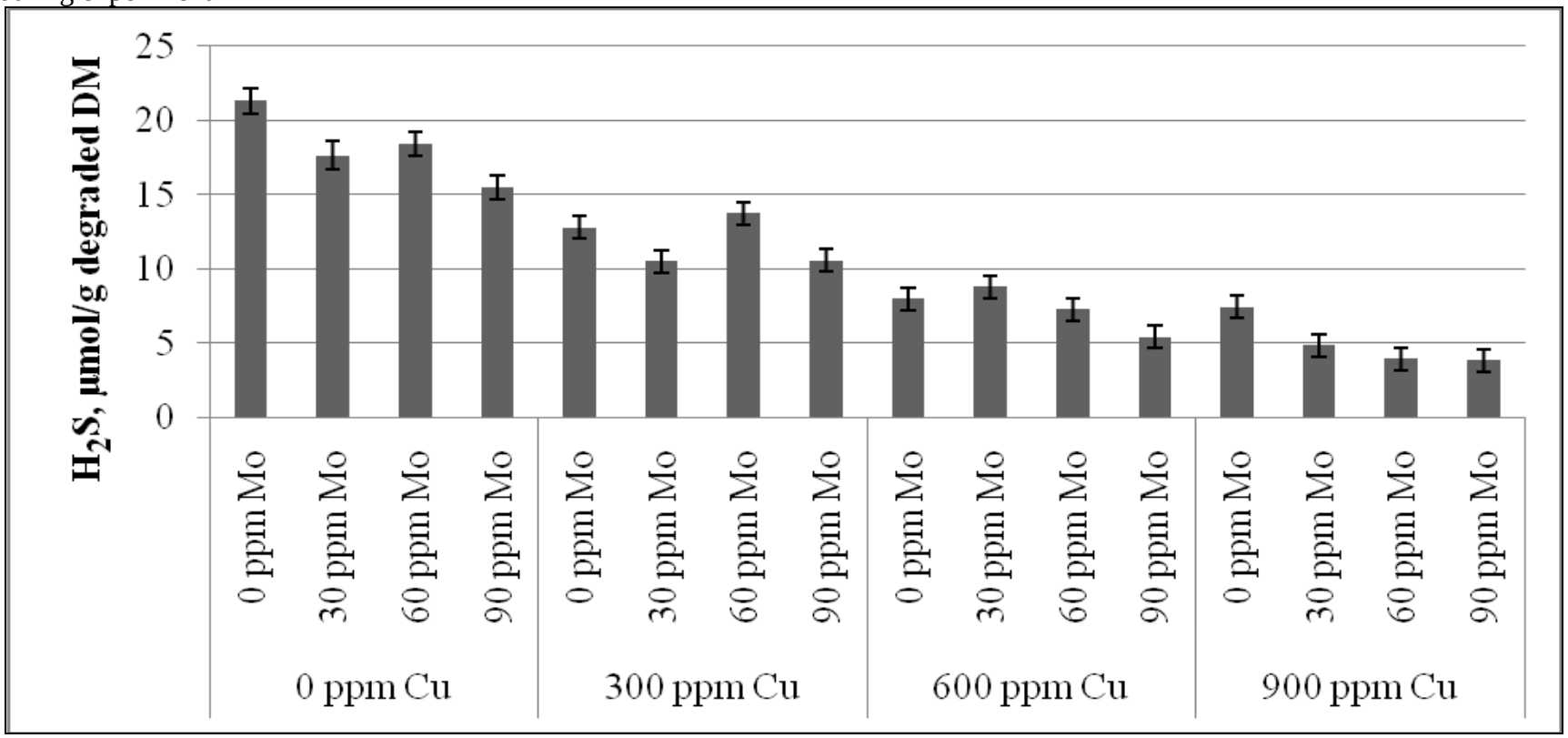
[1] Represents µmol of H_2S per g of degraded DDGS on a DM basis

[a, b, c] Means without common superscripts within a row differ

†† Molybdenum level has a significant interaction with Cu level ($P \leq 0.05$)

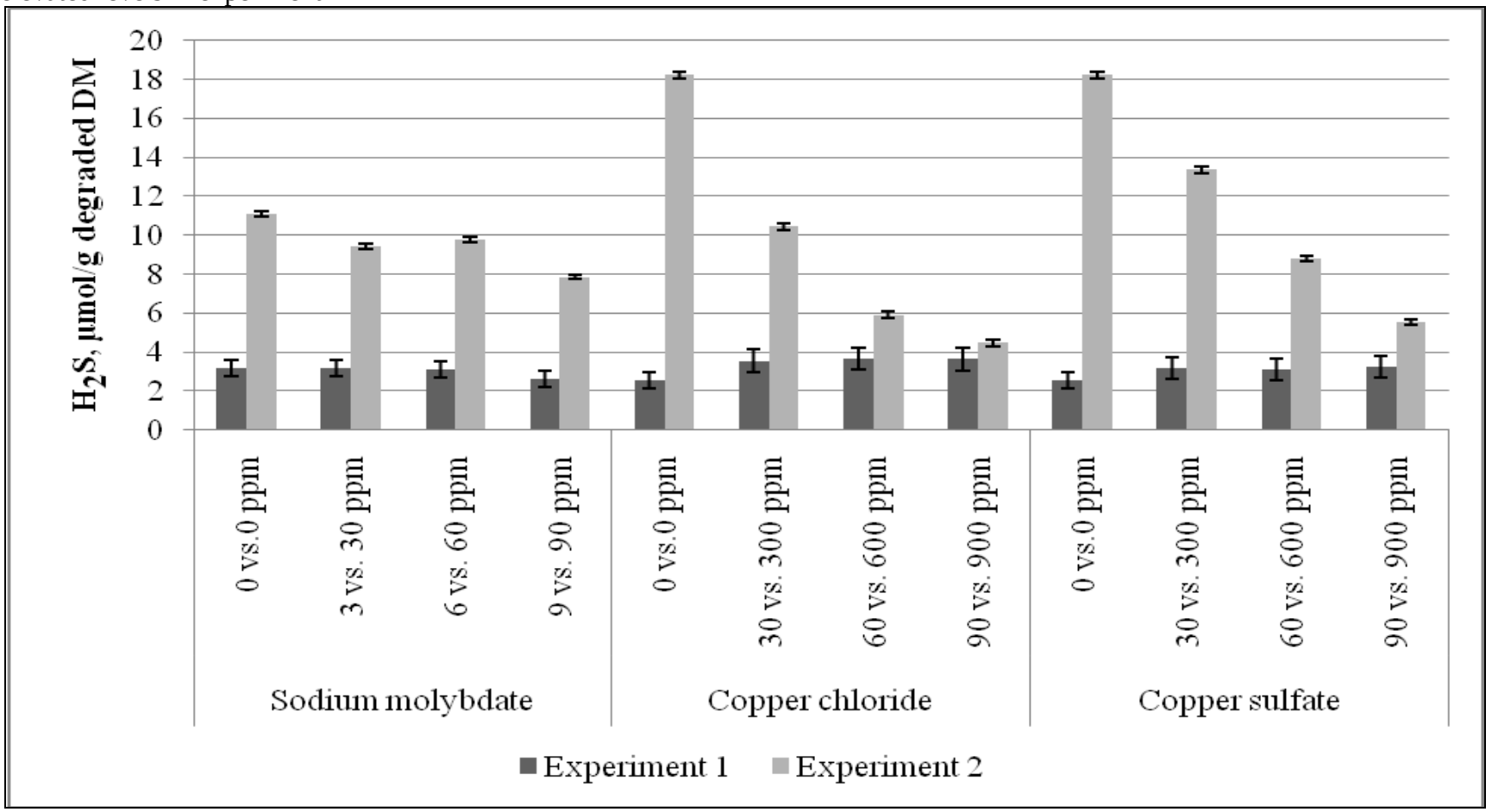
*** Copper sources differ ($P \leq 0.01$)

Figure D.4 Two-way interaction of molybdenum level by copper level for hydrogen sulfide adjusted for dry matter degradation during experiment 2



* Copper level represents an average from both Cu sources ($CuCl_2$ and $CuSO_4$)

Figure D.5 Comparison of hydrogen sulfide adjusted for dry matter degradation with the treatment levels from experiment 1 to the elevated levels in experiment 2



* Experiment 1 treatments were 0, 3, 6, and 9 ppm Mo from Na_2MoO_4 and 0, 30, 60, and 90 Cu from $CuCl_2$ and $CuSO_4$; experiment 2 treatments were increased 10-fold

DISCUSSSION

In-vitro laboratory work was conducted to determine appropriate combination of Mo and Cu that could be added to diets containing high levels of DDGS as a strategy to mitigate H_2S emissions without depressing ruminal function. Hydrogen sulfide concentrations showed high variance between experiments when comparing untreated vessels (Figure D.6). This may be an artifact of time that the rumen fluid was extracted from the cannulated cow. Kung et al. (2000) reported that volatile-fatty acid (**VFA**) concentrations and S production may be dependent on time of ruminal fluid collection relative to the last feeding. The pH within the rumen may offer further evidence of H_2S production as collection for experiment 2 was conducted within 4 h of feeding, which had a rumen fluid pH of 5.63. Whereas in experiment 1, rumen fluid was collected approximately 8 h post feeding and had a rumen fluid pH of 6.24. Another explanation why H_2S production was low in experiment 1 for untreated vessels compared to experiment 2 may possibly be due to the different methods used to bubble gas into alkaline water. In experiment 1, a 1:1 ratio using 10 ml of gas to 10 ml of pH 10 water was used; whereas in experiment 2, total gas withdrawn from the vessels were bubbled into 10 ml of pH 8 water. Similar studies by others used a 1:1 ratio of gas to pH 8 water (Siegal, 1965; Kung et al., 2000; Vanness et al., 2009). According to Cord-Ruwisch (1985) H_2S is still capable of volitilization in solution below a pH of 10. Within our lab, a comparison between gas bubbled in pH 8 water and pH 12 water was conducted with blank vessels, vessels containing 0.5 g of DDGS subsrate, and vessels with subsrate including treatment of 1000 ppm of Mn. The pH 12 water increased H_2S production 43.6% in the blank vessels, 47.8% with the DDGS substrate, and 39.2% with substrate treated with 1000 ppm Mn (data not shown).

In experiment 1, levels of emitted H_2S were low relative to other studies. A study from Kelzer et al. (2010) conducted an experiment using aproximately 0.7 g DM substrate consisting of 81% DGS that was incubated for 24 h at 39° C, producing H_2S at 2.23 and 1.13 µg/ml or 69.7 and 35.3 nmol/ml concentration of sulfide, respectively. These results are in accord to experiment 2 of the current study. A study conducted by Kung et al. (2000) used a 375 mg substrate containing either 1.09% S or 0.29% S incubated for 24 h at 40° C and reported a production of H_2S at 4.3 and 2.0 µmol, respectively. Within the current study, the control (untreated) vessels for experiment 1 had the lowest levels of emitted H_2S at 0.55 µmol and the amount of H_2S produced from the control vessels for experiment 2 was 4.03 µmol. Smith et al. (2010) reported H_2S gas to be in the range of 27.6 and 35.9 µmol per g of fermented DM when 0.8% S in substrate was added to in-vitro fermentation vessels. This is greater than the untreated vessels from experiment 2 of the current study, as H_2S levels adjusted for DM degradation was 18.21 µmol/g. However, the experiments within the current study were incubated for 16 h compared to 24 h used from Kung et al. (2000), Kelzer et al. (2010), and Smith et al. (2010). The amount of S added as substrate within our study is between the levels reported from Kung et al. (2000) and similar to the 0.8% S in substrate level from Smith et al (2010) as our DDGS substrate contanied 0.7% S. Furthermore, Kung et al. (2000) reported similar differences in pH when using Mo as a inhibitory sulfide treatment and Kelzer et al. (2010) also reported a similar pH range within treatment using Mn at levels ranging from 500 to 2500 ppm. Smith et al. (2010) reported IVDMD to be approximately 70% when S was included at 0.2, 0.4, and 0.8% of the

substrate. In-vitro dry matter disappearance within our study was between 43-48% for both experiments.

The combination of Cu and Mo at elevated levels did mitigate H_2S emissions in-vitro from experiment 2 within the current study. However, these concentrations were above the dietary levels recommended by the NRC (2000) to feed to finishing cattle. When Mo was added at concentration < 10 ppm, no differences were seen compared to the untreated vessels. Copper treatment from both $CuCl_2$ and $CuSO_4$ < 100 ppm increased H_2S emissions in-vitro as indicated from experiment 1 of the current study. A live animal study will offer further insight on how Mo and Cu interact with S in the rumen or excreta from growing steers fed various levels of DGS that in-vitro work may not be able to determine at lower concentrations. Furthermore, use of environmentally controlled rooms will offer the opportunity to monitor several gases at once and determine if the use of Cu and Mo to reduce H_2S emissions may affect other hazardous gases as well.

SUPPLEMENTAL TABLES AND FIGURES

Table D.4 Dilutions of copper sulfate solution during experiment 1 and 2

	Copper determination in 1 L stock solution	
600 ppm:	[(Cu = 63.55 g-mol)/ ($CuSO_4$ + 5H_2O = 249.68 g-mol)] = 0.2543 Cu (600 mg·L^{-1})/ (0.2543 Cu)/ (1000 mg·g^{-1}) = 2.36 g·L^{-1}	
Concentration	Equation steps	Volume[2], ml
90 ppm:	(90 mg·kg^{-1})·(5·10^{-4} kg DDGS*) = 0.045 mg Cu per vessel (0.045 mg)/ (600 mg·L^{-1}) = 7.5·10^{-5} L per vessel (7.5·10^{-5} L)·(1000 ml·L^{-1}) =	0.075
60 ppm:	(60 mg·kg^{-1})·(5·10^{-4} kg DDGS*) = 0.03 mg Cu per vessel (0.03 mg)/ 600 mg·L^{-1}) = 5.0·10^{-5} L per vessel (5.0 ·10^{-5} L)·(1000 ml·L^{-1}) =	0.05
30 ppm:	(30 mg·kg^{-1})·(5·10^{-4} kg DDGS*) = 0.015 mg Cu per vessel (0.015 mg)/ 600 mg·L^{-1}) = 2.5·10^{-5} L per vessel (2.5·10^{-5} L)·(1000 ml·L^{-1}) =	0.025
0 ppm[1]:	-	-

[1] 0 ppm includes 0.1 ml of water to equal the volume of the treated serum bottles

[2] Total volume equaled 0.1 ml. Water was added to adjust for the difference

* DDGS = substrate

Table D.5 Dilutions of copper chloride solution during experiment 1 and 2

	Copper determination in a 1 L solution [(Cu = 63.55 g-mol)/ ($CuCl_2 + 2H_2O$ = 170.48 g-mol)] = 0.3728 Cu (600 mg·L^{-1})/ (0.3728 Cu)/ (1000 mg·g^{-1}) = 1.61 g·L^{-1}	
Concentration	Equation steps	Volume[2], ml
90 ppm:	(90 mg·kg^{-1})·(5·10^{-4} kg DDGS*) = 0.045 mg Cu per vessel (0.045 mg)/ (600 mg·L^{-1}) = 7.5·10^{-5} L per vessel (7.5·10^{-5} L)·(1000 ml·L^{-1}) =	0.075
60 ppm:	(60 mg·kg^{-1})·(5·10^{-4} kg DDGS*) = 0.03 mg Cu per vessel (0.03 mg)/ 600 mg·L^{-1}) = 5.0·10^{-5} L per vessel (5.0 ·10^{-5} L)·(1000 ml·L^{-1}) =	0.05
30 ppm:	(30 mg·kg^{-1})·(5·10^{-4} kg DDGS*) = 0.015 mg Cu per vessel (0.015 mg)/ 600 mg·L^{-1}) = 2.5·10^{-5} L per vessel (2.5·10^{-5} L)·(1000 ml·L^{-1}) =	0.025
0 ppm[1]:	-	-

[1] 0 ppm includes 0.1 ml of water to equal the volume of the treated serum bottles

[2] Total volume equaled 0.1 ml. Water was added to adjust for the difference

* DDGS = substrate

Table D.6 Dilutions of sodium molybdate solution during experiment 1 and 2

	Molybdenum determination in a 1 L solution [(Mo = 95.94 g/mol)/ ($Na_2MoO_4 + 2H_2O$ = 241.95 g/mol)] = 0.3965 Mo (60 mg·L^{-1})/ (0.3965 Mo)/ (1000 mg·g^{-1}) = 0.15 g·L^{-1}	
Concentration	Equation steps	Volume[2], ml
9 ppm:	(9 mg·kg^{-1})·(5·10^{-4} kg DDGS*) = 0.0045 mg Cu per vessel (0.0045 mg)/ (60 mg·L^{-1}) = 7.5·10^{-5} L per vessel (7.5·10^{-5} L)·(1000 ml·L^{-1}) =	0.075
6 ppm:	(6 mg·kg^{-1})·(5·10^{-4} kg DDGS*) = 0.003 mg Cu per vessel (0.003 mg)/ 60 mg·L^{-1}) = 5.0·10^{-5} L per vessel (5.0 ·10^{-5} L)·(1000 ml·L^{-1}) =	0.05
3 ppm:	(3 mg·kg^{-1})·(5·10^{-4} kg DDGS*) = 0.0015 mg Cu per vessel (0.0015 mg)/ 60 mg·L^{-1}) = 2.5·10^{-5} L per vessel (2.5·10^{-5} L)·(1000 ml·L^{-1}) =	0.025
0 ppm[1]	-	-

[1] 0 ppm includes 0.1 ml of water to equal the volume of the treated serum bottles

[2] Total volume equaled 0.1 ml. Water was added to adjust for the difference

* DDGS = substrate

Table D.7 Fermentation vessel arrangement for molybdenum and copper concentrations during experiment 2

Inclusion Levels	0 ppm Mo	3 ppm Mo[1]	6 ppm Mo[1]	9 ppm Mo[1]
0 ppm Cu (*$CuSO_4$*) →	Bottle 1 & 2	Bottle 3 & 4	Bottle 5 & 6	Bottle 7 & 8
30 ppm Cu (*$CuSO_4$*) →	Bottle 9 & 10	Bottle 11 & 12	Bottle 13 & 14	Bottle 15 & 16
60 ppm Cu (*$CuSO_4$*) →	Bottle 17 & 18	Bottle 19 & 20	Bottle 21 & 22	Bottle 23 & 24
90 ppm Cu (*$CuSO_4$*) →	Bottle 25 & 26	Bottle 27 & 28	Bottle 29 & 30	Bottle 31 & 32
0 ppm Cu[2] (*$CuCl_2$*) →	Bottle 33 & 34	Bottle 35 & 36	Bottle 37 & 38	Bottle 39 & 40
30 ppm Cu (*$CuCl_2$*) →	Bottle 41 & 42	Bottle 43 & 44	Bottle 45 & 46	Bottle 47 & 48
60 ppm Cu (*$CuCl_2$*) →	Bottle 49 & 50	Bottle 51 & 52	Bottle 53 & 54	Bottle 55 & 56
90 ppm Cu (*$CuCl_2$*) →	Bottle 57 & 58	Bottle 59 & 60	Bottle 61 & 62	Bottle 63 & 64

[1] Molybdenum was added using Na_2MoO_4

[2] Experiment 2 had a total of 4 untreated bottles and experiment 1 was conducted with 2

* Duplicate blanks would be bottles 65 & 66

Table D.8 Probability of an interaction affects between Mo and Cu levels with Cu source during experiment 1 and 2

	Experiment 1						
Item	Cu level	Cu source	Cu level × Cu source	Mo level	Mo level × Cu level	Mo level × Cu source	Mo level × Cu level × Cu source
H_2S[1], nmol/ml	< 0.01	< 0.01	0.37	0.80	0.36	0.42	0.57
H_2S, nmol	< 0.01	< 0.01	0.31	0.84	0.30	0.42	0.53
H_2S, µmol/g degraded DM	< 0.01	< 0.01	0.12	0.88	0.58	0.29	0.91
Total gas volume, ml	0.04	< 0.01	0.23	< 0.01	0.16	0.12	0.05
IVDMD, %	0.19	0.06	0.16	0.29	0.85	0.09	0.06
pH	< 0.01	< 0.01	0.26	0.01	0.04	0.06	0.20
	Experiment 2						
Item	Cu level	Cu source	Cu level × Cu source	Mo level	Mo level × Cu level	Mo level × Cu source	Mo level × Cu level × Cu source
H_2S, nmol/ml	< 0.01	0.03	0.52	< 0.01	< 0.01	0.71	0.95
H_2S, µmol	< 0.01	< 0.01	0.19	< 0.01	**0.02**	0.40	0.98
H_2S, µmol/g degraded DM	< 0.01	< 0.01	0.20	< 0.01	**0.03**	0.19	0.91
Total gas volume, ml	< 0.01	< 0.01	0.75	< 0.01	< 0.01	< 0.01	0.01
IVDMD, %	0.88	0.50	0.33	0.72	0.51	0.52	0.99
pH	0.55	< 0.01	0.71	0.56	0.97	0.13	0.97

[1] Hydrogen sulfide represents the concentration of the total sulfide gas trapped in alkaline solution

Figure D.6 Three-way interaction plot between copper source, copper level, and molybdenum level for in-vitro dry matter disappearance during experiment 1

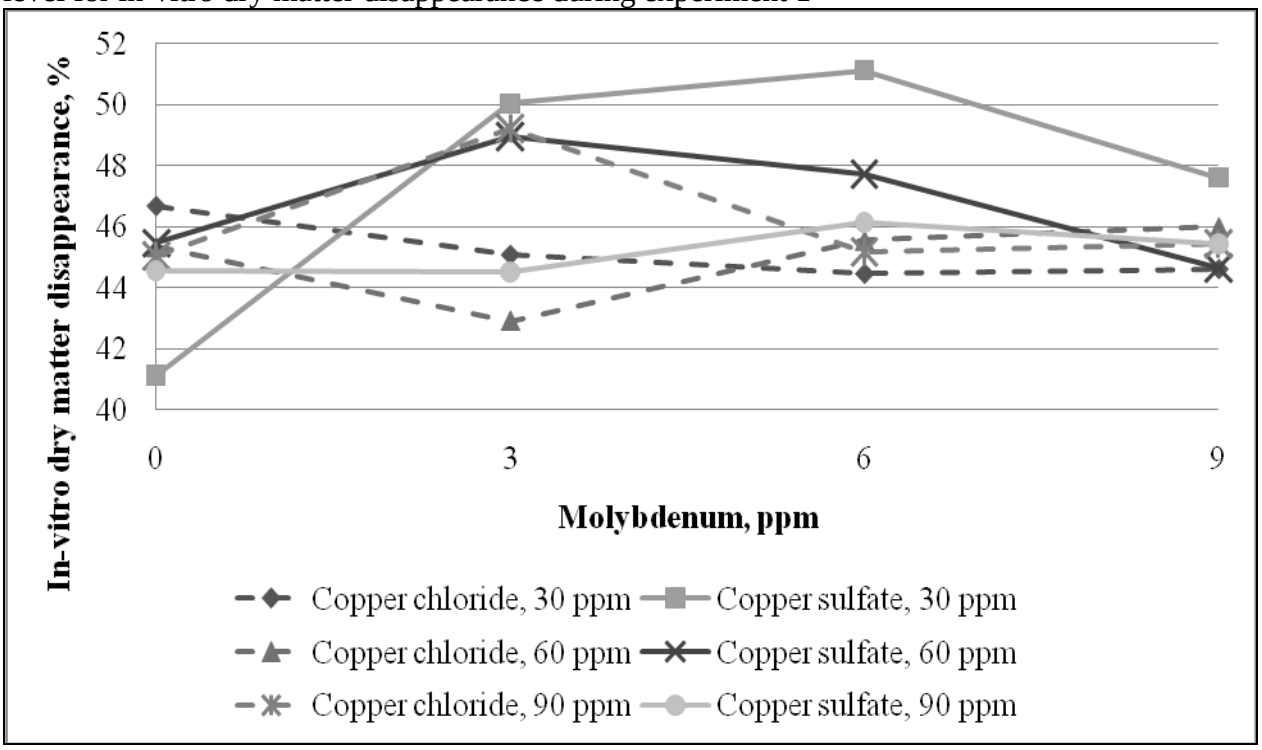


Figure D.7 Two-way interaction plot between copper source and molybdenum level for in-vitro dry matter disappearance during experiment 1

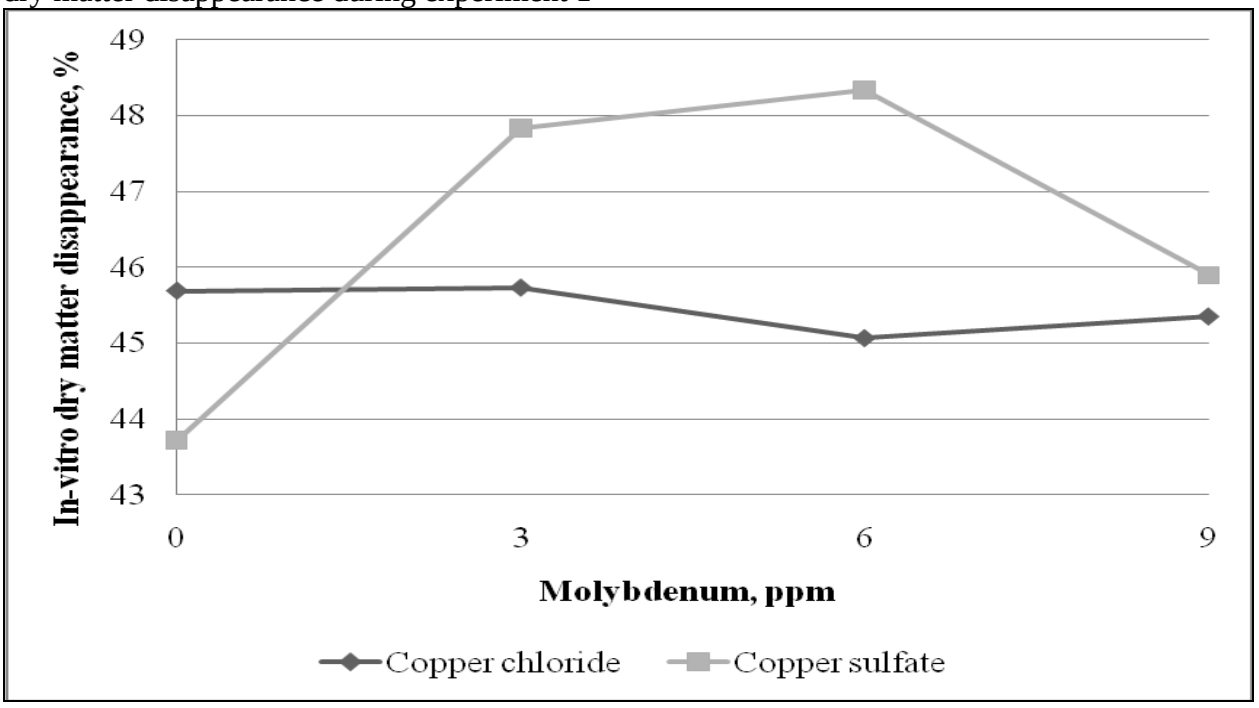


Figure D.8 Two-way interaction plot between copper source and molybdenum level for pH during experiment 1

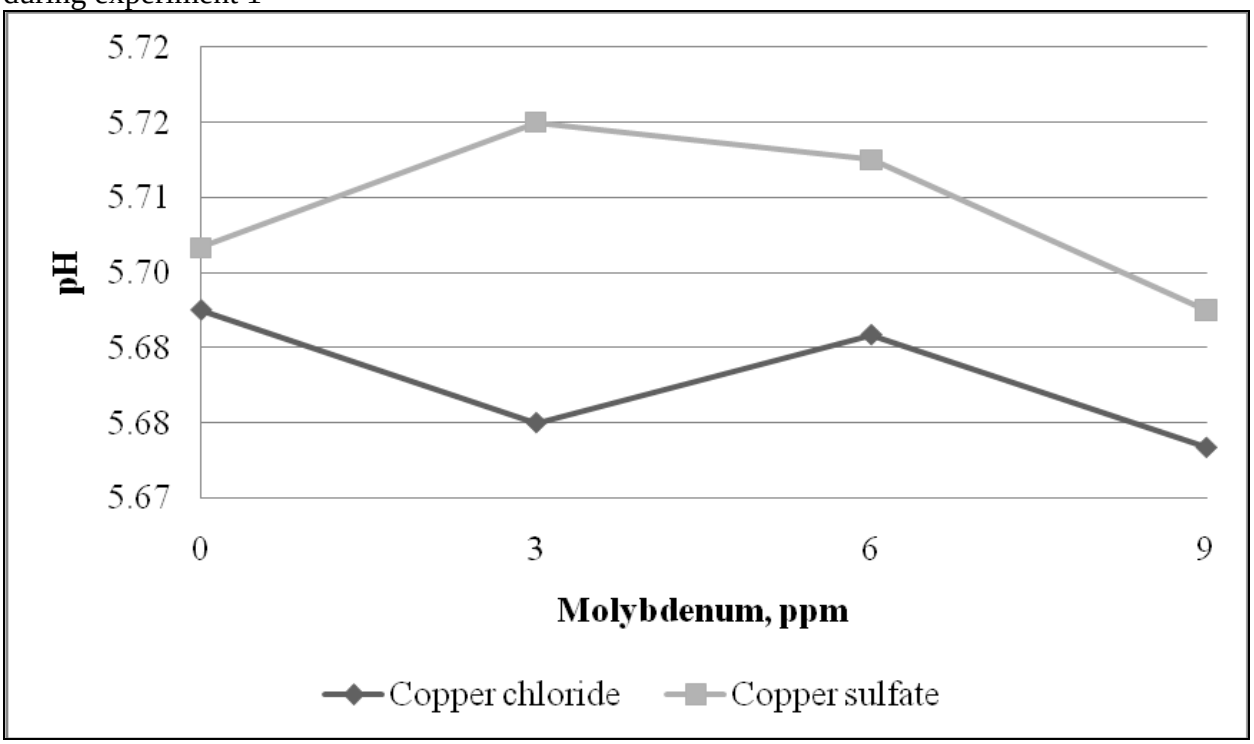


Figure D.9 Two-way interaction plot between copper level and molybdenum level for pH during experiment 1

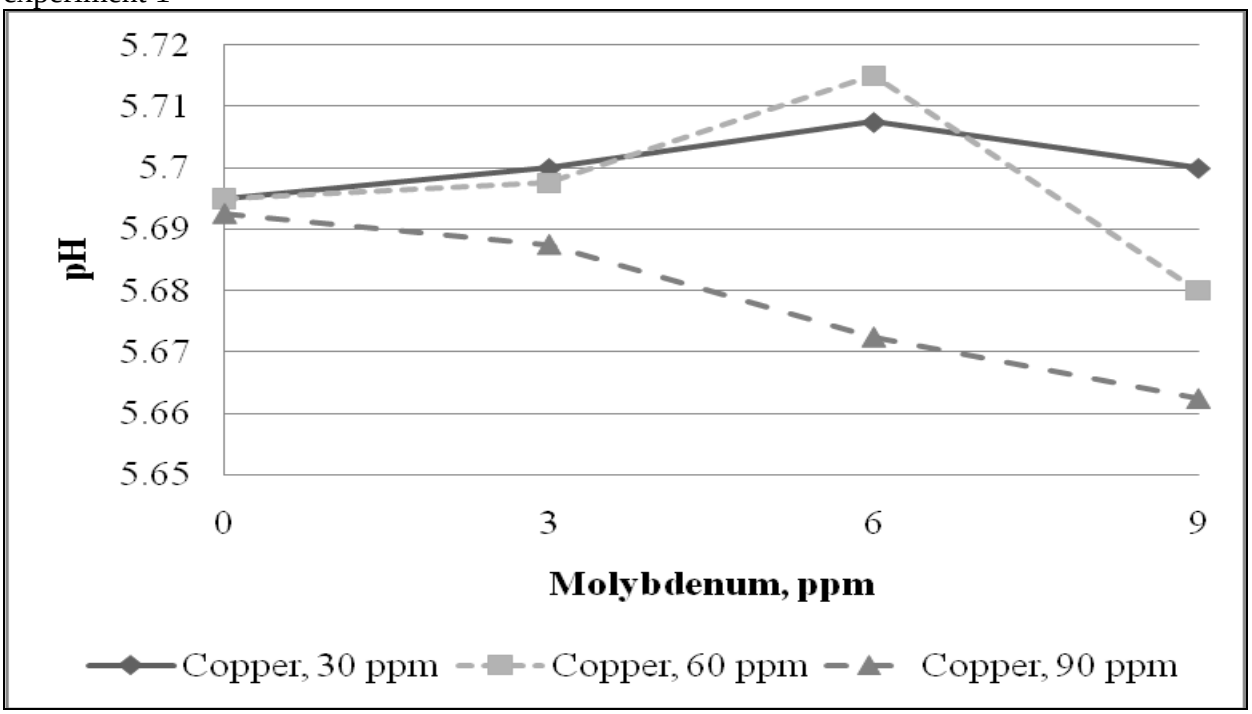


Figure D.10 Two-way interaction plot between copper level and molybdenum level for hydrogen sulfide during experiment 2

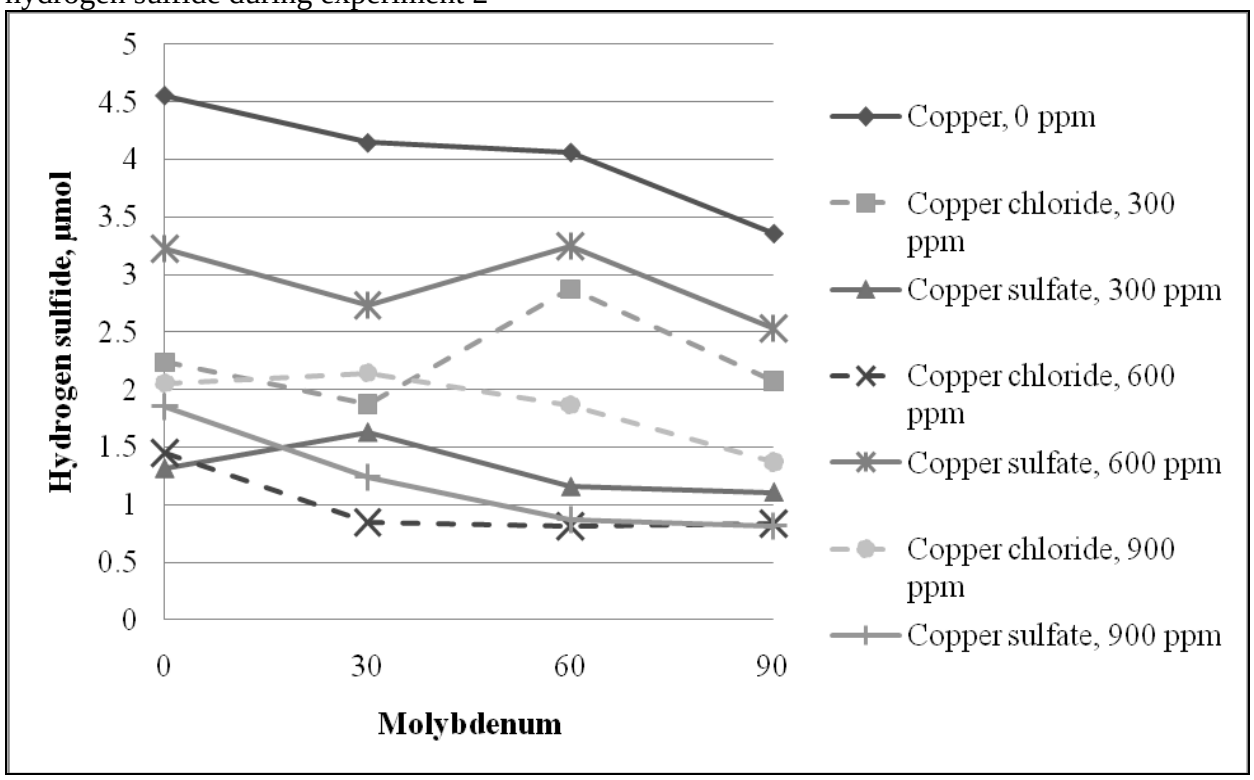


Table D.9 Preliminary results from an experiment with similar treatment levels as experiment 1

	Molybdenum, ppm						Contrast	
Item	0	3	6	9	SEM	*P*-value	Linear	Quadratic
H_2S, nmol†	714.0	678.4	747.8	645.4	37.5	0.26	0.42	0.38
H_2S, nmol/g	4.21^{a}	4.09^{a}	3.87^{ab}	3.09^{b}	0.24	< 0.01	< 0.01	0.17
IVDMD, %†	35.58^{a}	34.08^{a}	40.01^{b}	40.90^{b}	0.59	< 0.01	< 0.01	0.05
pH†	5.93^{a}	5.91^{a}	6.00^{b}	6.00^{b}	0.012	< 0.01	< 0.01	0.61

	Copper from $CuCl_2$, ppm							
Item	0	30	60	90	SEM	*P*-value	Linear	Quadratic
H_2S, nmol***	814.8^{a}	536.9^{b}	516.4^{b}	585.2^{b}	51.5	< 0.01	< 0.01	< 0.01
H_2S, nmol/g***	5.96^{a}	3.33^{b}	2.92^{b}	3.51^{b}	0.32	< 0.01	< 0.01	< 0.01
IVDMD, %***	29.36a	32.50^{b}	36.14^{c}	34.25^{bc}	0.86	< 0.01	< 0.01	< 0.01
pH***	5.80^{a}	6.06^{b}	6.08^{b}	6.04^{b}	0.016	< 0.01	< 0.01	< 0.01
	Copper from $CuSO_4$, ppm							
H_2S, nmol	814.8	821.4	885.7	714.6	61.2	0.15	0.25	0.07
H_2S, nmol/g	5.96^{a}	3.82^{b}	3.96^{b}	3.22^{b}	0.32	< 0.01	< 0.01	0.03
IVDMD, %	29.36^{a}	43.08^{b}	44.11^{b}	44.07^{b}	0.81	< 0.01	< 0.01	< 0.01
pH	5.80^{a}	5.85^{a}	5.96^{b}	5.93^{b}	0.016	< 0.01	< 0.01	0.01

$^{a, b, c}$ Means without common superscripts within a row differ ($P < 0.05$)

† Molybdenum level tends to express an interaction with Cu level ($P < 0.10$)

*** Copper sources differ ($P < 0.01$)

LITERATURE CITED

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Allen, J.D., and J.M. Gawthorne. 1987. Involvement of the solid phase of rumen digesta in the interaction between copper, molybdenum, and sulphur in sheep. Br. J. Nutr. 58:265.

Anderson, C.M. 1956. The metabolism of sulfur in the rumen of the sheep. N.Z. J. Sci. and Technol. 37(A):379.

Annison, E.F., and D.G. Armstrong. 1970. Page 422 in Physiology of Digestion and Metabolism in the Ruminant. A.T. Phillipson, ed. Oriel Press, Ltd., Newscastle-upon-Tyne, England.

Behlke, E.J., T.J. Klopenstein, T. Sanderson, and J.L. Miner. 2007. Replacement of forage with dried distillers grains reduces ruminal methane production. Pages 18- 21 in Nebraska Beef Cattle Reports. Animal Science Department. University of Nebraska- Lincoln.

Bird, P.R. 1972. Sulphur metabolism and excretion studies in ruminants IX. Sulphur, nitrogen, and energy utilization by sheep fed a sulphur-deficient and a sulphate-supplmentated roughage-based diet. Aust. J. Biol. Sci. 25:1073.

Block, R. J., R. Le Strange and G. Zweig. 1952. Page 55 in Paper Chromatography. Academic Press Inc.

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

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

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

Bouchard, R., and H.R. Conrad. 1972. Sulphur requirement of lactating dairy cows. I. Sulfur balance and dietary supplementation. J. Dairy Sci. Vol. 56. 10:1276-1282.

Bracht, J.P., and L. Kung, Jr. 1997. Inhibition of sulfide production in in vitro ruminal fermentations. Abstracts from the 24th Biennial Conf. on Rumen Function. Chicago, IL. 24:20.

Bray, A.C. 1969. Sulfur metabolism in sheep. III. The movement of blood inorganic sulfate across the rumen wall of sheep. Aust. J. Agr. Res. 20:749.

Brouwer, E. 1965. Energy Metabolism. Pages 441-443 in K.L. Blaxter, ed. Proc. 3rd Symposium. EAAP Pub. No. 11. Academic Press, London.

Buckner, C.D., T.L. Mader, G.E. Erickson, S.L. Colgan, D.R. Mark, V.R. Bremer, K.K. Karges, and M.L. Gibson. 2008. Evaluation of dry distillers grains plus solubles incusion on performance and economics of finishing beef steers. Prof. Anim. Sci. 24:404-410.

Bulgin, M.S., S.D. Stuart, and G. Mather. 1996. Elemental sulfur toxicosis in a flock of sheep. JAVMA. 208:1063-1065.

Bull, L.S., and J.H Vandersall. 1973. Sulfur source for in vitro cellulose digestion and in vitro ration utilization, nitrogen metabolism and sulfur balance. J. Dairy Sci. 56:106.

Cao, Z.J., J.L. Anderson, and K.F. Kalscheur. 2009. Ruminal degradation and intestinal digestibility of dried or wet distillers grains with increasing concentrations of condensed distillers solubles. J. Anim. Sci. 87:3013-3019.

Castillo, A.R., E. Kebreab, D.E. Beever, and J. France. 2000. A review of efficiency of nitrogen utilization in lactating dairy cows and its relationship with environmental pollution. J. Anim. Feed Sci. 9:1-32.

CEM Corporation. 1999. MARS 5: Microwave accelerated reaction system operation manual.

Chen, M.C., W.M. Benson, T.W. Perry, and M.T. Mohler. 1977. Effects of varying levels of processed distillers solubles and distillers grains with solubles on nitrogen and energy metabolism of beef steers. J. Anim. Sci. 44:859-866.

Cole, N.A., R.N. Clark, R.W. Todd, C.R. Richardson, A. Gueye, L.W. Greene, and K. McBride. 2005. Influence of dietary crude protein concentration and source on potential ammonia emissions form beef cattle manure. J. Anim. Sci. 83:722-731.

Cooling, F.B., III, C.L. Maloney, E. Nagel, J. Tabinowski, and J.M. Odom. Inhibition of sulfate respiration by 1,8-dihydroxyanthraquinone and other anthraquinone derivatives. Appl. Environ. Microbiol. 62:2999–3004.

Corah, L., and M. McCully. 2006. Declining quality grades: A review of factors reducing marbling deposition in beef cattle. Certified Angus Beef White Paper.

Cord-Ruwisch, R. 1985. A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria. J. Microbiological Methods. 4:33-36.

Crawford, G.I. 2007. Managing sulfur concentrations in feed and water. University of Minnesota Ext. 68th Minn. Nutr. Conf. Minneapolis, MN.

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

Depenbusch, B.E.,E.R. Loe, M.J. Quinn, M.E. Corrigan, M.L. Gibson, K.K. Karges, and J.S. Drouillard. 2008. Corn distillers grains with solubles derived from a traditional or partial fractionation process: Growth performance and carcass characteristics of finishing feedlot heifers. J. Anim. Sci. 86:2338-2343.

Depenbusch, B.E., C.M Coleman, J.J Higgins, and J.S. Drouillard. 2009. Effects of increasing levels of dried corn distillers grains with solubles on growth performance, carcass characteristics, and meat quality of yearling heifers. J. Anim. Sci. 87:2653-2663.

Donham, K.J., J. Yeggy, and R.R. Dague. 1988. Production rates of toxic gases from liquid swine manure: implications for workers and animals in swine confinement buildings. Biol. Wastes. 24:161-173.

Erickson, G.E., and T.J. Klopenstein. 2001. Managing N inputs and the effect on losses following excretion in open-dirt feedlots in Nebraska. The Sci. World J. 1(S2):830-835.

Fahey, G.C, Jr., and L.L. Berger. 1988. The Ruminant Animal: Digestive Physiology and Nutrition by D.C. Church. Pages 278-279 in Carbohydrate Nutrition of Ruminants. Prentice-Hall, Inc. Englewood Cliffs, NJ.

Farlin, S.D. 1981. Wet distillers grains for finishing cattle. Anim. Nutri. Health. 36:35-36.

Ferrell, C.L. 1988. The Ruminant Animal: Digestive Physiology and Nutrition by D.C. Church. Pages 250-254 in Energy Metabolism. Prentice-Hall, Inc. Englewood Cliffs, NJ.

Fron, M.J., J.A. Boling, L.P. Bush, and K.A. Dawson. 1990. Sulfur and nitrogen metabolism in the bovine fed different forms of supplemental sulfur. J. Anim. Sci. 68:543-552.

Garcia-Lopez, P.M., L. Kung Jr., and J.M. Odom. In vitro inhibition of microbial methane production by 9,10 anthraquinone. J. Anim. Sci. 74:2276–2284.

Gengelback, G.P., J.D. Ward, and J.W. Spears. 1994. Effect of dietary copper, iron, and molybdenum on growth and copper status of beef cows and calves. J. Anim. Sci. 72:2722-2727.

Gerber, D.B., K.M. Manci, M.A. Veenhuizen, and G.C. Shurson. 1991. Ammonia, carbon monoxide, carbon dioxide, hydrogen sulfide, and methane in swine confinement facilities. Compendium on Continuing Education for the Practicing Veterinarian. 13:1483-1488.

Gill, R.K., D.L. VanOverbeke, B. Depenbusch, J.S. Drouillard, and A. DiCostanzo. 2008. Impact of beef cattle diets containing corn sorghum distillers grains on beef color, fattly acid profiles and sensory attributes. J. Anim. Sci. 86:923-935.

Goering, H.K., and P.J. Van Soest. 1970. Forage and fiber analysis. Agricultural Handbook no. 379. U.S. Dept. of Agriculture.

Gooneratne, S.R., A.A. Olkowski, R.G. Klemmer, G.A. Kessler, and D.A.Christensen. 1989. High sulfur related thiamine deficiency in cattle: a field study. Can. Vet. J. 30:139-146.

Gordon, C.M., J.S. Drouillard, J. Gosch, J.J. Sindt, S.P. Montgomery, J.N. Pike, T.J. Kessen, M.J. Supizio, M.F. Spire, and J.J. Higgins. 2002. Dakota gold brand dried distillers grains with solubles: Effects on finishing performance and carcass characteristics. Pages 27-29 in Kansas State University Cattlemen's Day Report of Progress. No. 890. Kansas State University. Manhatten, KS.

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

Gould, D.H., D.A. Dargatz, F.B. Garry, and D.W. Hamar. 2002. Potentially hazardous sulfur conditions on beef cattle ranches in the United States. JAVMA. 221:673-677.

Green, F.H.Y., S. Schurch, G.T. DeSanctis, J.A. Wallace, S. Cheng, and M. Prior. 1991. Effects of hydrogen sulfide exposure on surface properties of lung surfactant. Am. Pathol. Soc. No. 5. 70:1943-1949.

Guang-hui, Y.U., X.U. Xiao-jun, and H.E. Pin-jing. 2007. Isolates identification and characteristics of microorganisms in biotrickling filter and biofilter system treating H_2S and NH_3. J. Environ. Sci. 19:859-863.

Gueye, A., C.R. Richardson, J.H. Mikus, G.A. Nunnery, N.A. Cole, and L.W. Greene. 2003. Effects of dietary crude protein on serum and urine nitrogen in feedlot steers. J. Anim. Sci. 81(Suppl. 1):210. (Abstr.)

Gunn, P.J., A.D. Weaver, R.P. Lemenager, D.E. Gerrard, M.C. Claeys, and S.L. Lake. 2009. Effects of dietary fat and crude protein on feedlot performance, carcass characteristics, and meat quality in finishing steers fed differing levels of dried distillers grains with solubles. J. Anim. Sci. 87:2882-2890.

Hamsell, A.M., Y. Xue, C.D. Robinson, M.A. Canalizo-Hernández, R.G. Marvin, R.A. Kelly, A. Mondragón, J.E. Penner-Hahn, and T.V O'Halloran. 2010. Tetrathiomolybdate inhibits copper trafficking proteins through metal cluster formation. Science. Vol. 327.

Hays, F.L., E. Goret, H.D. Johnson, and L. Hahn. 1972. Hydrogen sulfide (H_2S) exposure in ruminants. J. Anim. Sci. 35:189.

Henry, W.A. 1900. Dried distillery grains compared to oats. Feeds and Feeding 2nd ed. 421. Morrison Publishing Co., Ithica, NY.

Hession, A.O., L. Kung, Jr., and C. A. Bessett. 1995. Altering ruminal fermentation and

inhibiting ruminal sulfide production with 9,10 Anthraquinone (AQ). 23rd Rumen Function Conf.. Abstract # 12. Chicago, IL.

Holt, S.M., and R.H. Pritchard. 2004. Composition and nutritive value of corn co-products from dry milling ethanol plants. South Dakota State Beef Report.

Hooser, S.B., W. Van Alstine, M. Kiupel, and J. Sojka. 2000. Acute pit gas (hydrogen sulfide) poisoning in confinement cattle. J. Vet. Invest. 12:272-275.

Huber, J.T., N.O. Price, R.W. Engel. 1971. Response of lactating dairy cows to high levels of dietary molybdenum. J. Anim. Sci. 32:364-367.

Innova Airtech Instruments A/S. Measuring gases with the help of microphones– photoacoustic effect. Innova Airtech Instruments A/S. 2750 Ballerup, DK.

Intergovernmental Panel on Climate Change. 2007. Climate Change 2007: The Physical Science Basis. Page 33 in Technical Summary. Cambridge University Press. Cambridge, United Kingdom and New York, NY, USA.

Johnson, W.H., R.D. Goodrich, and J.C. Meiske. 1971. Metabolism or radioactive sulfur from elemental sulfur, sodium sulfate and methionine by lambs. J. Anim. Sci. 32:778.

Johnson, D.E., and G.M. Ward. 1996. Estimates of animal methane emissions. Environmental Monitoring and Assessment. 42:133-141.

Jones, J.G., B.M. Simon, and S. Gardner. 1982. Factors affecting methanogenesis and associated processes in the sediments of a stratified eutrophic lake. J. Can. Microbiol. 128:1-11.

Jones, R.D., and M.A. Hood. 1980. Effects of temperature, pH, salinity, and inorganic nitrogen on rate of ammonium oxidation by nitrifiers isolated from wetland environments. Microb. Ecol. 6:339-347.

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

Kaiser, R.M. 2005. Variation in composition of distillers wet grains with solubles. Pages 191-197 in the proceedings of the 4-State Dairy Nutrion and Management Conf. Dubuque, IA.

Kalshear, K.F., and A.D. Garcia. 2008. New horizons in ethanol byproducts. Proc. Dairy Calf and Heifer Assoc. South Dakota State University. Brookings, SD.

Kambara, S., T. Takarada, M. Toyoshima, and K. Kate. 1995. Relation between functional forms of coal nitrogen and NOx emissions from pulverized coal combustion. Pages 1247-1253 in Fuel. Elsevier Science Ltd. Vol. 74. No. 9. Great Britain.

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

Karkalas, J. 1985. An improved enzymic method for the determination of native and modified starch. J. Sci. Food Agric. 36:1019-1027.

Kelzer, J.M., T.D. Maddock, M. Ruiz Moreno, A. DiCostanzo, G.I. Crawford. 2010. Use of manganese oxide to attenuate negative effects on high sulfur concentrations in distillers grains-based finishing rations. Pages 40-52 in Proc. Minnesota Beef Cow Calf Days. University of Minnesota, St. Paul.

Kleinschmit, D.H., J.L. Anderson, D.J. Schingoethe, K.F. Kalscheur, and A.R. Hippen. 2007. Ruminal and intestinal degradability of distiller's grains plus solubles varies by source. J. Dairy Sci. 90:2909-2918.

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

Koeln, L.L., and J.A. Paterson. 1987. Nitrogen balance and amino acid disappearance from the small intestine in calves fed soybean meal-, toasted soybean meal- or corn gluten meal-supplemented diets. J. Anim. Sci. 63:1258-1266.

Koelsch, R.K., B.L. Woodbury, D.E. Stenburg, D.N. Miller, and D.D. Schulte. 2004. Hydrogen sulfide concentration in vicinity of beef cattle feedlots. Applied Engineering in Agric. 20(1):77-85.

Koziel, J.A., C. Lingshuang, D.W. Wright, and S.J. Hoff. 2006. Solid-phase microextraction as a novel air sampling technology for improved, GC-olfactometry-based assessment of livestock odors. J. Chromatogr. Sci. 44:451-457.

Kroger, T. J., D.M. Wulf, K.E. Tjardes, K.S. Mateo, T.E. Engle, C.L. Wright, and R.J. Maddock. 2004. The influence of feeding various levels of wet distiller's grains on carcass quality, carcass composition, and fatty acid profile of finishing steers. Proc. 57th Recip. Meats Conf.

Kung, L. Jr., L. A.O. Hession, and J.P. Bracht. 1998. Inhibition of sulfate reduction to sulfide by 9,10-anthraquinone in in-vitro ruminal fermintations. J. Dairy Sci. 81:251-2256.

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

Kung, L. Jr. 2008. Burping can be dangerous if you are a ruminant: issues with high sulfur diets. Proc. Four state dairy nutrition and management conference. University of Delaware. Newark, DE.

Kurihara, M., T. Magner, R.A. Hunter, and G.J. McCrabb. 1999. Methane production and energy partition of cattle in the tropics. Brit. J. Nutri. 81:227-234.

Larson, E.M., R.A. Stock, T.J. Klopenstein, M.H. Sindt, and R.P. Huffman. 1993. Feeding value of wet distillers byproducts for finishing ruminants. J. Anim. Sci. 71:2228-2236.

Lesperance, A.L., V.R. Bohman, and J.E. Oldfield. 1985. Interaction of molybdenum, sulfate and alfalfa in the bovine. J. Anim. Sci. 60:791-802.

Liamleam, W., and A.P. Annachhatre. 2007. Electron donors for biological sulfate reduction. Biotechnol. Adv. 25:452-463.

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. JAVMA. 213:1599-1604.

Luebes, R.E., K.R. Davis, and A.E. Laag. 1974. Diurnal fluctuation and movement of atmospheric ammonia and related gases from dairies. J. Environ. Qual. 3: 265-269.

Lyford, S.R. Jr., and D.C. Church 1988. Page 28 in The Ruminant Animal: Digestive Physiology and Nutrition. Prentice-Hall, Inc. A division of Simon & Schuster. Englewood Cliffs, NJ.

Mackie, R.I., P.G. Stroot, and V.H. Vareal. 1998. Biochemical identification and biological origin of key odor components in livestock waste. J. Anim. Sci. 76:1331-1342.

Marcilese, N.A., C.B. Ammerman, R.M. Valsecchi, B.G. Dunavant, and G.K. Davis. 1970. Effect of dietary molybdenum and sulphate upon urinary excretion of copper in sheep. J. Nutr. 100:1399-1405.

Mason, K. A conspectus of research on copper metabolism and requirements of man. J. Nutri. 109:1990.

Mauricio, R.M., F.L. Mould, M.S. Dhanoa, E. Owen, K.S. Channa, and M.K. Theodorou. 1999. A semi-automated in vitro gas production technique for ruminant feedstuff evaluation. Anim. Feed Sci. and Tech. 79:321-330.

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

McAllister, M.M. D.H. Gould, and D.W. Hamar. 1992. Sulphide-in-duced polioencephalomalacia in lambs. J. Comp. Pathol. 106:267-278.

McBride, K.W., L.W. Greene, N.A. Cole, F.T. McCullum, and M.L. Galyean. 2003. Nitrogen and phosphorus utilization in beef cattle fed three levels of dietary crude protein and three degradable intake protein/undegradable intake protein ratios. J. Anim. Sci. 81(Suppl. 1): 73. (Abstr.)

Mills, C.F., I. Bremner, and T.T. El-Gallad. 1978. Mechanisms of the Mo/sulphur antagonism of copper utilization by ruminants. Pages 150-158 in Kirchgessner, M., ed. Trace elements in man and animals. 3 Freising: Institut Für Ernährungsphysiologie.

Mills, C.F. 1980. Metabolic interactions of copper with other trace elements. Page 46 in Mill C.F., ed. Biological roles of copper. Amsterdam: Excerpta Medica.

Merck Veterinary Manual. 1991. Whitehouse Station, New Jersey: Merck and CO., Inc. 614-616.

Misselbrook, T.H., J.M. Powell, G.A. Broderick, and J.H. Grabber. 2005. Dietary manipulation in dairy cattle: laboratory experiments to assess the influence on ammonia emissions. J. Dairy Sci. 88:1765-1777.

Mohammed, N., R. Onodera, and M.M. Or-Rashid. 2003. Degradation of tryptaphan and related indolic compounds by ruminal bacteria, protozoa and their mixture in vitro. Amino Acids. 24:73-80.

Monares, S.W., and J.D. Ingle Jr. 2010. Adapting the methylene blue sulfide detection method for use in a microfluidic in situ monitoring device. For a degree of Honors Baccalaureate of Science in Chemistry. Presented May 25, 2010. Department of Chemistry, Oregon State University, Corvallis, OR 97331.

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

Nagaraja, T.G. 1995. Ionophores and antibiotics in ruminants. Pages 173-204 in Wallace, R.J., A. Chesson (eds.). Biotechnology in Animal Feeds and Animal Feeding. VCH, NY.

NRC. 1996. Nutrient requirements of beef cattle. 7th ed. Natl. Acad. Press. Washington, DC.

NRC. 1998. Nutrient requirements of swine. 10th rev. ed. Natl. Acad. Press. Washington, DC.

NRC. 2000. Nutrient requirements of beef cattle. 7th ed. Natl. Acad. Press. Washington, DC.

NRC. 2005. Mineral tolerance of animals. 2nd ed. Natl. Acad. Press. Washington, D.C.

Odom, J.M., and R. Singleton Jr. 1993. Pages 189-219 in The sulfate-reducing bacteria: contemporary perspectives. Springer-Verlag Inc., New York.

Oliveira, L. A., C. Jean-Balin, S. Komisarczuk-Bony, A. Durix, and C. Durier. 1997. Microbial thiamin metabolism in the rumen simulating fermenter (RUSITEC): the effect of acidogenic conditions, a high sulfur level and added thiamin. Brit. J. Nutr. 78:599-

613.

Olkowski, A.A., S.R. Gooneratne, C.G. Rousseaux, and D.A. Christensen. 1992. Role of thiamine status in sulphur induced polioencephalomalacia in sheep. Vet. Sci. 52:78-85.

Oremland, R.S., and D.G. Capone. 1988. Use of specific inhibitors in biogeochemistry and microbial ecology. Adv. Microbiol. Ecol. 10:285-383.

Osweiler, G.D., T.L. Carson, W.B. Buck, and G.A. Van Gelder. 1985. Clinical and diagnostic veterinary toxicology. Pages 369-377 in the 3rd ed. Kendall/Hunt. Dubuque, IA.

Phillip, L.E., J.G. Buchanan-Smith, and W.L. Grovum. 1980. Effect of ensiling whole plant corn on voluntary intake, rumen fermentation, retenetion time and rate of digestion in steers. J. Anim. Sci. 51:1003-1010.

Pickrell, J. 1991. Hazards in confinement housing- gases and dusts in confined animal houses for swine, poultry, horses and humans. Vet. Hum. Toxicol. 33:32-39.

Pollution Prevention and Abatement Handbook. 1998. Pages 223-226 in Project Guidelines: Pollutants. World Bank Group. Washington.

Powers, W.J., H.H. Van Horn, A.C. Wilkie, C.J. Wilcox, and R.A. Nordstedt. 1999. Effects of anaerobic digestion and additives to effluent or cattle feed on odor and odorant concentrations. J. Anim. Sci. 77:1412-1421.

Powers, W.J., B.J. Kerr, and K.J. Stalder. 2006. Influence of corn co-products on air emissions and nutrient excretions from grow-finish swine. Final report submitted to the National Pork Board 7/1/06. Michigan State University. East Lansing, MI.

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

Rausch, K.D., R.L. Belyea. 2006. The future of coproducts from corn processing. Appl. Biochem. Biotechnol. 128:47–86.

Renewable Fuels Association. 2006. Renewable fuels outlook 2006. Renewable Fuels Association, Washington, DC.

Renewable Fuels Association. 2010. Renewable fuels outlook 2010. Renewable Fuels Association, Washington, DC.

Roeber, D.L., R.K. Gill, and A. DiCostanzo. 2005. Meat quality responses to feeding distiller's grains to finishing Holstein steers. J. Anim. Sci. 83:2455-2460.

Rosser, R.A., C.E. Polan, P.T. Chandler, and T.L. Bibb. 1971. Effects of whey components and methionine analog on bovine milk fat production. J. Dairy Sci. 54:1807.

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

Salisbury, M.W., C.R. Krehbiel, T.T. Ross, C.L. Schultz, and L.L. Melton. 2004. Effects of supplemental protein type intake, nitrogen balance, and site, and extent of digestion in whiteface wethers consuming low-quality grass hay. J. Anim. Sci. 82:3567-3576.

Sawyer C.N., and P.L. McCarty. 1978. Chemistry for Sanitary Engineers. McGraw-Hill, New York, NY.

Sawyer, J.E. 2001. Nitrogen fertilizer and swine manure application to soybean. Presented at the 2001 Inegrated Crop Management Conf. Ames, IA.

Schmidt, I., O. Sliekers, M. Schmid, I. Cirpus, M. Strous, E. Bock, J. Gijs Kuenen, and M.M. Jetten. 2002. Aerobic and anaerobic ammonia oxidizing bacteria- competitors or natural partners? Microb. Ecol. 39:175-181.

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

Shurson, J., M. Whitney, and R. Nicolai. 1998. Nutritional manipulation of swine diets to reduce hydrogen sulfide emissions. Pages 219-240 in Proc. 59th Minnesota Nutri. Conf. IPC Tech. Symp., Bloomington, MN.

Siegel, L.M. 1965. A direct microdetermination for sulfide. Anal. biochem. 11:126-132.

Slyter, L. L.,W. Chapula, and R. R. Oltjen. 1988. Response to elemental sulfur by calves and sheep fed purified diets. J. Anim. Sci. 66:1016–1027.

Smart, M.E., R. Cohen, D.A. Christensen, and C.M. Williams. 1986. The effect of sulphate removal from the drinking water on the plasma and liver copper and zinc concentrations of beef cows and their calves. Can. J. Anim. Sci. 66:669–680.

Smith, S.B., and J.D. Crouse. 1984. Relative contributions of acetate, lactate and glucose to lipogenesis in bovine intramuscular and subcutaneous adipose tissue. J. Nutr. 114:792.

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

Spais, A.G., T.K. Lazaridis, and A.K. Agiannidis. 1968. Studies on sulfur metabolism in sheep in association with copper deficiency. Res. Vet. Sci. 9(4):337-344.

Spieghs, M.J., and V.H. Varel. 2009. Nutrition excretion and odorant production in manure from cattle fed corn wet distillers grains with solubles. J. Anim. Sci. 87:2977-2984.

Spoelstra, S.F., 1980. Origin of objectionable odorous components in piggery wastes and the possibility of applying indicator components for studying odor development. Agric. Environ. 5 (3):241–260.

Starks, P.B., W.H. Hale, U.S. Garrigus, and R.M. Forbes. 1953. The utilization of feed nitrogen by lambs as affected by elemental sulfur. J. Anim. Sci. 12:480.

Stein, H. H., C. Pedersen, M. L. Gibson, and M.G. Boersma. 2006. Amino acid and energy digestibility in ten samples of distillers dried grain with solubles by growing pigs. J. Anim. Sci. 84:853–860.

Stock, R.A., J.M. Lewis, T.J. Klopfenstein, and C.T. Milton. 2000. Review of new information on the use of wet and dry milling feed by-products in feedlot diets. J. Anim. Sci. 78(E-Suppl.) Accessed March 9, 2011. http://www.asas.org/symposia/9899proc/0924.pdf.

Suttle, N.F., and A.C. Field. 1983. Effects of dietary supplements of thiomolybdates on copper and molybdenum metabolism in sheep. J. Comp. Pathol. 93:379.

Suttle, N.F., P. Abraham, I. Thornton. 1984. The role of a soil x dietary sulfur interaction in the impairment of copper absorption by ingested soil in sheep. J. Agri. Sci. Camb. 103:81-86.

Suttle, N.F. 1991. The interactions between copper, molybdenum, and sulphur in ruminant nutrition. Annu. Rev. Nutr. 11:121-140.

Tanimoto, Y., M. Tasaki, K. Okamura, M. Yamaguichi, and K. Minami. 1989. Screening growth inhibitors of sulfate-reducing bacteria and their effects on methane formation. J. Fermen. Bioeng. 68:353-359.

Tchnobanoglous, G., and F.L. Burton. 1991. 3rd ed. Wasterwater engineering, treatment, disposal and reuse. McGraw-Hill Series in Water Resources and Environmental Engineering. New York, NY: Metcalf & Eddy, Inc.

TEI-17C Operator's Manual. 2001. Reduced nitrogen (NH_3) monitoring with the TEI model 17C analyzer. Sec. 2.22.1. Rev. No. 0.

Thermo Fisher Scientific Inc. Hydrogen sulfide and sulfur dioxide analyzer, model 450*i*. Pulsed fluorescence gas analyzer. 27 Forge Parkway, Franklin, MA, USA.

Thistlethwayte, D.K.B. 1972. The control of sulfides in sewerage systems. Ann Arbor Science Publishers Inc. Ann Arbor, MI.

Tyrrell, H.F., A.C.G. Brown, P.J. Reynolds, G.L. Haaland, D.E. Bauman, C.J. Peel, and W.D. Steinhour. 1988. Effect of bovine somatotropin on metabolism of lactating dairy cows: energy and nitrogen utilization as determined by respiration calorimetry. J. Nutr. 118:1024-1030.

Underwood, E.J. 1977. Trace elements in human and animal nutrition (4th ed.). Academic Press, San Fransisco, CA.

US Environmental Protection Agency. 1994. InternationalAnthropogenic Methane Emissions: Estimates for 1990. EPA230-R-93-010. Washington, DC. Office of Policy, Planning and Evaluation, US EPA.

US Environmental Protection Agency. 2000. Emission Facts: Average Annual Emissions and Fuel Consumption for Passenger Cars and Light Trucks. Office of Transportation and Air Quality. NVFEL Library. Ann Arbor, MI, USA.

Vander Pol, K.J., G.E. Erickson, T.J. Klopenstein, M.A. Greenquist, and T. Robb. 2006. Effect of dietary inclusion of wet distillers grains on feedlot performance of finishing cattle and energy value relative to corn. Nebraska Beef Cattle Report. MP88-A:51-53.

Van Nevel, C., and D. Demeyer. 1996. Lipolysis and biohydrogenation of soyabean oil in the rumen in vitro: inhibition byantimicrobials. J. Dairy Sci. 78:2797–2806.

Vannes, S.J., T.J. Klopenstein, G.E. Erickson, and K.K. Karges. 2009a. Sulfur in distillers grains. Nebraska Beef Report. University of Nebraska. Lincoln, NE. MP92:79-80.

Vannes, S.J., C.D. Buckner, V.R. Bremer, N.A. Ahern, T.J. Klopenstein, and G.E. Erickson. 2009b. Hydrogen sulfide gas levels post feeding. Nebraska Beef Report. University of Nebraska. Lincoln, NE. MP92:84-85.

Van Soest, P.J. 1982. Nutritional Ecology of the Ruminant. O & B Books, Inc., Corvallis, OR.

Varel, V.H., J.E. Wells, E.D. Berry, M.J. Spiehs, D.N. Miller, C.L. Ferrell, S.D. Shackelford, and M. Koohmaraie. 2008. Odorant production and persistence of Escherichia coli in manure slurries from cattle fed zero, twenty, or sixty percent wet distillers grains with solubles. J. Anim. Sci. 86:3617-3627.

Westermann, P., and B.K. Ahring. 1987. Dynamics of methane production, sulfate reduction, and denitrification in a permanently waterlogged alder swamp. Applied and Environ.Microbiol. 53: 2554–2559.

Whanger, P.D., and G. Matrone. 1970. Effects of sulfur deficiency on metabolism in sheep. In: O.H. Muth (ed.) Symp. Sulfur in Nutrition. AVI Publishing Co. Inc. Westport, CT.

Woods, M., and J. Mason. 1987. Spectral and kinetic studies on the binding of trithiomolybdate to bovine and canine serum albumin in vitro: The interaction with copper. J. Inorg. Biochem. 30:261.

Xue, S.K., S. Chen, and R.E. Hermanson. 1998. Measuring ammonia and hydrogen sulfide emitted from manure storage facilities. ASAE. Vol. 41. 4:1125-1130.

Zaun, J.A., J.L. Hatfield, Y.S. Do, A.A. DiSpirito, D.A. Laird, and R.L. Pfeiffer. 1997. Characterization of volatile organic emissions and wastes from a swine production facility. J. Environ. Qual. 26:1-20.

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