DISSERTATION

APPLICATION OF YEAST TECHNOLOGY IN RUMINANT NUTRITION

Submitted by

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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY KELCEY L. SWYERS ENTITLED APPLICATION OF YEAST TECHNOLOGY IN RUMINANT NUTRITION BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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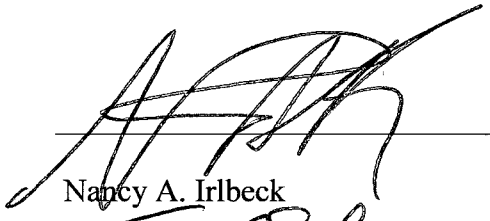

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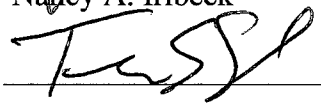

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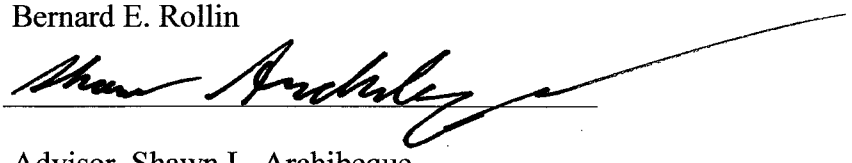

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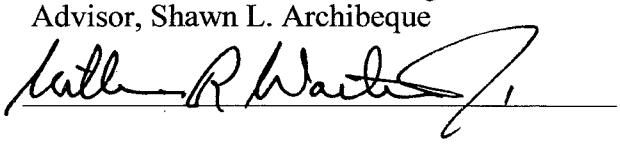

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ABSTRACT OF DISSERTATION

APPLICATION OF YEAST TECHNOLOGY IN RUMINANT NUTRITION

This research aimed to evaluate two types of yeast technology applications that are commercially available to feed in the diets of beef cattle; these two applications are dried distiller's grains with solubles (**DDGS**) and yeast culture (**YC**). First, DDGS were offered as a protein and energy supplement to pregnant range cows to evaluate performance, and nitrogen and trace mineral status of cows and calves (Exp. 1), then YC was fed as a natural alternative to an ionophore to heavy-weight yearling beef steers fed finishing diets consisting of 19.7% DDGS to evaluate growth performance, carcass merit, cost of gain (**COG**) (Exp. 2), and fecal prevalence of the foodborne pathogen, *E. coli* O157:H7 (Exp. 3). In Exp. 1, supplementing the diets of pregnant beef cows maintained on dormant winter range with DDGS improved cow performance, had limited impact on nitrogen or mineral status, and was more economical to feed than the control diet (standard range cubes). In Exp. 2, we found that feeding YC may improve carcass characteristics of steers finished at lower end weights, which could result in fewer days on feed; however, there was no advantage on growth performance or COG. Lastly, Exp. 3 indicated that feeding YC decreased, but did not eliminate, fecal shedding of *E. coli* O157:H7 upon initial application.

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CHAPTER I

LITERATURE REVIEW

Introduction[a]

Commercial Applications of Yeast. The aim of this review is to discuss the application and importance of applying yeast (fungi) technology in animal nutrition. Yeast technology has become increasingly advanced in the last 25 years, deepening our understanding of the industrial potential of the organism. Commercially, the ability to engineer and manipulate the fermentative capacity of yeast has generated heightened interest in potential applications of the organism. Yeasts have been exploited commercially for baking, brewing, ethanol production, probiotics, nutritional benefits, and advances in biotechnology. Nearly 90,000 fungal species have been described; however, some estimate that 1.5 million species may exist with global distribution from polar to tropical regions (Willey et al., 2008). This diversity in species is characterized, in part, by the natural environment in which each unique strain inhabits, which include (but are not limited to) plants, soil, fresh and marine water, skin surfaces, and in the intestinal tracts of warm-blooded animals (where they may live symbiotically or as parasites). This diversity allows different strains of *Saccharomyces* to perform different tasks. It is this diversity that interests researchers in animal nutrition to identify and develop strains of *Saccharomyces* that can positively alter the fermentation that occurs in the digestive tract and the subsequent end-products that could potentially be beneficial to host animals such as beef cattle. Among the various strains of yeasts that have been identified, strains of *S. cerevisiae* dominate most commercial yeast-oriented industries and will be the predominant species of interest for this review.

The most common applications of yeast technology in animal nutrition are: direct-fed microorganism applications (active dry yeast or yeast culture), production of

[a] This literature review was prepared following Journal of Animal Science style and form.

brewing and grain distilling by-products (distillers dried grains and brewers dried grains), and as a direct source of dietary crude protein (brewers dried yeast) (AAFCO, 2007). The focus of this discussion is to review the recent advances in yeast technology in animal nutrition, specifically as it pertains to beef nutrition, with the focus regarding the former two applications. Therefore, the application of yeast (and other yeast –associated by-products) as a source of dietary crude protein will not be further discussed in this review; consequently, the reader is directed to a review by Westerndorf and Wohlt (2002). Additionally, this discussion aims at the application of yeast technology in ruminants, specifically in beef production systems; however, in some instances discussion of other animal models and production systems are both relevant and necessary toward constructing a comprehensive review on the subject matter and will therefore be included.

Beef production is the largest single segment of the agricultural industry in the U.S., accounting for 31 % of America's farms (NCBA, 2008). In 2008, the meat industry (excluding poultry) reported gross sales of approximately $66 billion, with beef accounting for more than 52% of dollars spent at retail (NCBA, 2008). It is well established that feed costs attribute to the greatest economic expense to the beef cattle producer. Small improvements in feed efficiency cannot only positively affect animal performance, but also profitability. Therefore, solutions that can increase feed efficiency and improve production, while also minimizing cost and without cuasing detriment to the health and welfare of animals, is of critical interest and demand by the beef industry.

Yeast Physiology. The basic concepts of yeast ecology, biochemistry, physiology, and molecular biology have been previously reviewed (Trivedi et al. 1986; Madigan et

al., 2000; Bai et al., 2007; Fleet, 2008; Willey et al., 2008) and will be relied upon for this discussion. Yeasts are eukaryotic microorganisms, or fungi, that are considered to be facultative anaerobes (being able to exist with or without oxygen and can obtain energy by fermentation). Because yeasts are classified as eukaryotic cells, similar to the cells in mammalian systems, they can be widely used for mammalian genetic and disease-related research. Like mammalian systems, *Saccharomyces* reproduce sexually, however they are unique in that this sexual reproduction is achieved by budding and the bearing of spores. Yeast cell growth and proliferation is dependent on availability of substrate (or growth-limiting nutrients) and the ability of daughter cells to reach a 'critical' size before new buds can form. Between each cell cycle, or a new generation of daughter cells, there is an inherent lag that exists between the mother cell and its daughter cell. Each daughter bud that separates can grow into new yeast cells and some group together to form colonies.

The most commonly used substrate for industrial yeast propagation and fermentation are sugars such as glucose, sucrose (from either beet or cane molasses), galactose, and maltose. Yeasts also require some nitrogenous compounds (from either amino acid or ammonia sources) for *de novo* amino acid and protein synthesis. Yeasts release exogenous hydrolytic enzymes that are able to digest nitrogenous substrates externally. While yeasts usually perform aerobic fermentation, yeasts found in the rumen of cattle are obligately anaerobic fungi. The principal end-products of fungal anaerobic fermentation in the rumen consist of amino acids (arginine, cysteine, and glutamine), short chain fatty acids (**SCFA**; acetate, propionate, and butyrate), ethyl alcohol (ethanol; $\mathbf{C_2H_6O}$), carbon dioxide ($\mathbf{CO_2}$), methane ($\mathbf{CH_4}$), ammonia ($\mathbf{NH_3}$), and hydrogen ($\mathbf{H_2}$). Of

primary interest to this discussion is the fermentative action of yeasts in the production of ethanol. The main metabolic pathway involved in the production of ethanol is glycolysis through which one molecule of glucose is metabolized and two molecules of pyruvate are produced. It has been estimated that 30 to 65% of total cellular proteins present in yeast cells are glycolytic enzymes (Willey et al., 2008). Under anaerobic conditions, pyruvate is further reduced to ethanol with the release of CO_2. Interestingly, the survival of certain yeast strains is largely determined by their different susceptibilities to an increasing concentration of ethanol during fermentation.

Yeast Technology as a Direct-Fed Microbial Application

Probiotics. In recent years, researchers have become more acutely aware of the normal flora that inhabit the gastro intestinal (**GI**) tract of mammals and the benefits that the flora can exert upon the host. Of primary interest is to understand, characterize, and optimize the fermentative action of the normal flora for the benefit of mammalian species. An accelerated thrust of research efforts has focused on the enhancement the normal flora (across mammalian, avian, and aquatic species) through the application of microbial feed additives; commonly referred to as 'probiotics.'

Probiotics were first recognized by Metchnikoff (1907) who speculated that the longevity of Bulgarian peasants was achieved by organisms, present in the fermented milk (yogurt) they were consuming, that were inhibiting pathogens otherwise causing disease. Later, Rettger and Chaplin (1921) confirmed that bacteria, identified as *Lactobacillus acidophilus,* in the yogurt acted as an antibiotic. Over four decades later, Lilley and Stillwell (1965) were the first to define probiotics as, "substances secreted by

one organism that stimulates the growth of another." More recently, probiotics have been defined as, "microorganisms that beneficially affect the host animal by providing intestinal microbial balance" (Fuller, 1989). The U.S. Office of Regulatory Affairs of the Food and Drug Administration (FDA, 1995) and the Association of American Feed Control Officials (AAFCO, 2007) have narrowed the definition of probiotics to, "a source of live, naturally occurring microorganisms" (Yoon and Stern, 1995) and require animal feed manufacturers to use the term 'direct-fed microorganisms' (**DFM**).

A review of the literature indicates an array of positive effects achieved through DFM application. In ruminants, microbial cultures have been shown to decrease the incidence of ruminal acidosis (Ghorbani et al., 2002), improve feed efficiency and daily gain in beef cattle (Ware et al., 1988), potentially replace or reduce the use of antibiotics in neonatal and stressed calves (Abu-Tarboush et al., 1996), and enhance milk production in dairy cows (Komari et al., 1999; Gomez-Basauri et al., 2001). In poultry, DFM supplementation has improved egg production, feed consumption, feed conversion, eggshell thickness, and yolk color, and has decreased yolk cholesterol (Mohan et al., 1995; Yeo and Kim, 1997; Li et al., 2006). Weaned pigs offered DFM have shown reduced incidence, severity and duration of diarrhea, and reduced fecal shedding of *Salmonella* spp. (Casey et al., 2007). Mature horses supplemented with DFM had increased digestibility of copper, zinc, and iron (Swyers et al., 2008). Probiotics offered to humans reduced serum cholesterol level and colon cancer, and improved calcium absorption, vitamin synthesis, and lactose tolerance (Fuller, 1989; Tannock, 1999), as well as reduced the incidence of diarrhea in children (Van Niel et al., 2002).

Most commercial DFM preparations are commonly comprised of bacterial species isolated from the GI tract of mammals. For that reason, it is important to understand and characterize the community of microorganisms that originate from and colonize the GI tract; these microorganisms are referred to as the "normal flora" (Parker, 1974). The makeup of the normal flora of an individual depends upon its species, age, sex, level of stress, and diet and consists of fungi, yeasts, protozoa, and some archaea, with bacteria comprising the largest component of the biomass (Todar, 2007). Due to a co-habitative relationship, the normal flora derives from the host a supply of nutrients, a stable environment, constant temperature, protection, and transportation whereas the host obtains from the normal flora certain nutritional metabolites, stimulation of the immune system, and exclusion of pathogens (Hungate, 1966; O'Sullivan et al., 2005). Within the intestinal lumen, different microenvironments exist; acidophiles will populate the proximal duodenum where acid secreted from the stomach is more persistent, while species less tolerant to low pH inhabit the rumen or distal colon. Some species thrive at the mucosal surface, while others are more stable in the crypts (Ward et al., 1990; Conway, 1995). The vast range of microbial species in the GI tract have specialized niches, varying along with their substrate specificity. In many cases, the metabolic end-products excreted or produced by one species can serve as a growth substrate for another. Consequently, the biomass of each species is directly correlated with the amount of substrate available (Gibson and Roberfroid, 1995). For that reason, it has been suggested that probiotics are likely host species-specific (due to specificities of varying species' diets) and is not likely that all DFM preparations would exhibit benefits across-species (Gibson and Fuller, 2000). However, it could be said that our understanding of

mammalian normal flora and its mode of action has been virtually unexplored. It has been estimated that only a fraction (< 1 %) of microbial species have been recovered through isolation and cultivation, suggesting that our understanding of the GI microbial ecosystem, based on the few strains we have identified, is likely misleading (Amann et al., 1995; Daly et al., 2001; Weese, 2004).

Certain fungal cultures, such as strains of *Saccharomyces* and *Aspergillus*, have been evaluated for their DFM properties. For example, some strains of *Saccharomyces* and *Aspergillus* can either be offered as 'yeast culture' (**YC**), "the dried product composed of yeast and the media on which it was grown, dried in such a manner as to preserve the fermenting activity of the yeast" (AAFCO, 2007), or as 'active dry yeast' that contains "not less than 15 billion live yeast cells per gram" (AAFCO, 2007). Commercial products vary widely in both the strains of yeast used and the number and viability of yeast cells present. Given the AAFCO definition, it seems that YC would best serve either as a protein supplement or as a 'prebiotic.' Prebiotics have been described as "non-digestible [by the host animal] food ingredients (typically in the form of carbohydrates) that beneficially affect the host by stimulating growth and/or activity of certain bacterial components of the intestinal micro flora" (Gibson and Roberfroid, 1995). When YC is offered as a prebiotic, it can provide soluble growth factors such as organic acids, B vitamins, and amino acids that can stimulate bacterial growth in the natural flora of ruminants (Callaway and Martin, 1997).

In a review of ruminant and non-ruminant literature, the most consistent response to offering YC in the animal's diet is an increase in cecal and ruminal bacterial yield, specifically of cellulolytic species (Glade and Biesik, 1986; Hall et al., 1990; Glade,

1991a, 1991b, 1992; Callaway and Martin, 1997, Nagaraja et al., 1997; Glade and Sist, 1988; Medina et al., 2002). Reports range from 5 to 40 times greater numbers of cellulolytic species present in ruminants fed high-roughage diets (Hillman et al., 1985; Newbold et al., 1993). It is theorized that YC promotes a higher pH environment (Dawson et al., 1990), which is more favorable toward the growth of cellulolytic bacteria such as *Fibrobactor succinogenes, Ruminococcus albus,* and *Ruminococcus flavefasciens* (Miller-Webster et al., 2002). Williams et al. (1991) also suggested that increased fiber digestion seen in ruminants is mediated by an increase in ruminal pH.

Mode of Action. The mode of action explaining increased rumen pH is likely due to an increased number of lactate-utilizing bacteria (**LUB**) when *S. cerevisiae* is offered (Edwards 1991; Williams et al., 1991; Girard et al., 1993). An accumulation of lactate decreases rumen pH (Krehbeil et al.; 2003). Whereas amylolytic (or lactic-acid producing bacteria) utilize starch and produce lactic acid as a by-product of fermentation, LUB convert lactate to propionate (Roxas; 1980). In short, lactate produced from starch fermentation can be converted to propionate by LUB (Hobson and Stewart, 1997). The predominant LUB of the rumen are *Selenomonas ruminantium*, *Propionibacterium acnes* and *Anaerovibrio lipolytica* (Hobson and Stewart, 1997). In addition to ruminants, this 'buffering effect' by LUB has also been noted due to the supplementation of YC in the diets of horses (Medina et al., 2002), ponies (Moore and Newman, 1993), and growing pigs (van Heugten et al., 2003). More specifically, Medina et al. (2002) reported that *S. cerevisiae* increased pH and decreased lactate in cecal and colon samples of mature fistulated horses fed high-starch diets. While the digestive morphology is quite different between ruminants and non-ruminants, it has been observed that some of the same

cellulolytic bacteria common in the rumen are also present in the horse cecum (Julliand et al., 1999). Whereas *R. flavefaciens* is the most predominant cellulolytic bacteria found in the cecum, *F. succinogens* is the main cellulolytic species in the rumen (Julliand et al., 1999, Michaelet-Doreau et al., 2002).

Increasing the pH in the GI environment has not only been implicated with increased fermentative capacity of cellulolytic bacteria, but also with increased mineral retention and the flow of microbial cell protein to the small intestine in ruminants (**MCP**; Dawson et al., 1990; Wohlt et al., 1991; Martin and Nisbet, 1992; Cole et al., 1992; Newbold et al., 1996; Miller-Webster et al., 2002). More specifically, Carro et al. (1992a; 1992b) observed a significant increase in the synthesis of MCP and the subsequent increase in flow of undegraded dietary protein from the rumen of dairy cows fed *S. cerevisiae*. In agreement, Erasmus et al. (1992) noted an association with the feeding of *S. cerevisiae* and an increased amount of MCP leaving the rumen and amount of amino acids entering the small intestine. However, some ruminant studies have reported no beneficial effects with YC supplementation (Quionez et al., 1988; Erdman and Sharma, 1989; Arambel and Kent, 1990), with one study reporting an induced inflammatory response when YC was supplemented in high-concentrate diets of growing feedlot steers (Emmanuel, et al., 2007). In contrast, a series of equine studies (Glade and Biesik, 1986; Glade and Campbell Taylor, 1990; Glade, 1991a; Glade, 1991b; Kim et al. 1991) demonstrated that *S. cerevisiae* increased digestibilities of DM, NDF and ADF, increased N retention, improved milk quality in mares, and increased growth in foals. More recently, others (Morgan et al., 2007; Jouany et al., 2008) have reported improved DM, NDF, ADF, and CP digestibilities in horses receiving YC, and hypthosized

(although has not yet been measured) that this was likely due to stimulation of microbial cellulolytic activity in the hindgut.

In theory, improving fiber digestion should promote increased feed efficiency and performance particularly in growing and lactating animals. Hinman et al. (1998) reported that the addition of YC in the finishing diet of cross-bred steers increased both ADG and G : F. Wohlt et al. (1991) observed increased digestibilities of protein and cellulose, increased DMI, and higher average milk yield in Holstein cows offered 10 $g \cdot cow^{-1} \cdot d^{-1}$ of *S. cerevisiae* in diets consisting of a 1 : 1 ratio of corn silage : grain (DM-basis) plus 0.9 kg/d of hay over an 18-wk period. More recently, Schingoethe et al. (2004) reported increased feed efficiency in mid-lactation Holstein cows fed 60 $g \cdot cow^{-1} \cdot d^{-1}$ of YC in diets containing 28 % corn silage, 21 % alfalfa hay, and 51 % concentrate (DM-basis) over a 12-wk period. However, others (Piva et al., 1993; Robinson, 1997; Robinson and Garrett, 1999; Dann et al., 2000) have reported varied results when *S. cerevisiae* has been offered to dairy cows. For example, while Dann et al. (2000) reported increased DMI in Jersey cows during the transition and postpartum periods, supplementing the diet with YC offered no advantage in milk yield. The specificities for appropriate (or optimal) dose and duration of supplementation for each specific strain have not been fully elucidated to obtain a specific response; this likely attributes to the variation in responses observed between these studies.

Yeast Technology in the Production of Distiller's Grains

Corn to Ethanol. Fuel ethanol has become a booming industry in those countries where agricultural and economic conditions are favorable; first in Brazil in 1975,

followed by the USA in 1978, and then more recently, in Canada (Wheals et al., 1999). The primary organism used for ethanol production is *S. cerevisiae,* which can efficiently and economically ferment monosaccharides that become available (from chemical and mechanical manipulation) from feedstuffs (such as corn, sorghum, barley, wheat, sugarcane, sugar beets, and other lignocellulosic crops or wood fibers), on a large-scale, into fuel ethanol (Fortman et al., 2008; Lyons et al., 1995; Laluce 1991). The ethanol produced is separated from other fermentation solubles using distillation. Guiroy et al. (2007) indicated that one bushel of corn will yield 10.2 L of ethanol, 8 kg of dried distiller's co-product, and 8 kg of CO_2. In other words, the net yield is approximately two-thirds of the original kernel converted into ethanol and CO_2, and the other third into feed co-products. A typical stoichiometry from maltose to glucose to ethanol might be:

$$C_{12}H_{22}O_{11} + H_2O \rightarrow 2\ C_6H_{12}O_6 \rightarrow 4\ C_2H_5OH + 4\ CO_2 + \text{heat}$$

The production of ethanol and CO_2 are produced to tight specifications, therefore any constituent variability that could exist in ethanol and CO_2 should be considered negligible. Consequently, most, if not all the variability found from ethanol processing would be expected to be magnified in the feed by-product, creating a challenge to nutritionists trying to formulate consistent diets (Guiroy et al., 2007).

Reviews by Wheals et al. (1999) and Rausch and Belyea (2005) were used extensively in this discussion to describe the following commercial processes in which grain can be converted to ethanol. Those processes that will be discussed include: annexed distillation (typical of sugar-ethanol combined manufacturing plants), dry- and wet-milling, and dry-grind corn processing. Each process relies on different technologies and results in different co-products. For example, in annexed distillation, cane juice,

extracted from sugar cane (which contains 38-60 % total sugars on a DM-basis), can be heated up to 110 °C to reduce microbial contamination, decanted (to pour off without disturbing the sediment), sometimes concentrated by evaporation, and then fermented by yeast to make ethanol. On the other hand, if the sucrose crystals have been removed from the cane juice by centrifugation for the purpose of sugar refinery, then syrup or molasses would remain, which contains up to 65 % sugars on DM-basis. This syrup can also be immediately used by *S. cerevisiae* to ferment ethanol. The primary co-product from annexed distillation that is marketed to livestock producers is molasses. A normal cane molasses usually has a water content of 17–25%, a sugar content (sucrose, glucose, fructose) of 45–50% and polysaccharides (dextrin, pentosans, polyuronic acids) containing 2–5% (Najafpour and Poi Shan, 2003).

In contrast, crop grains such as corn, barley, or wheat (following the removal of the germ and bran), can be processed by either wet- or dry-milling procedures. In wet-milling, the purpose is to isolate and recover starch (used to produce glucose, high fructose corn syrup, or ethanol). The grains are steeped (soaked or saturated) in water containing sulphur dioxide (used to to swell and soften the kernels and to leach solubles from the germ). After 24 to 48 h, the steepwater (or liquor) is drawn off and concentrated. The steepwater contains 45 to 50 % total protein and can be used to form synthetic amino acids. Then, the germ is separated from the remaining corn fractions and is washed to remove any starch. The germ is then further refined and filtered to make corn oil, and any remaining solubles can be sold as a by-product feed for livestock known as corn germ meal. The other corn fractions are collected, ground, slurried, and then the starch fraction is separated from the gluten via centrifugation. The starch fraction is

typically sold as a purified product (i.e. glucose or high fructose corn syrup) or can also be further used for microbial fermentation to produce ethanol for beer. The co-products from wet-milling that can be marketed to livestock producers are corn gluten meal (**CGM**) and corn gluten feed (**CGF**; Blasi et al., 2001). The purpose of dry-milling is to physically separate corn components (fat from the germ is separated from the endosperm) for the purpose of human consumption. The kernel is swelled to increase the moisture from 15 to 22 %, and then the kernel goes through a series of degermination, aspiration, and gravity separation to separate the pericarp (bran), germ and endosperm fragments. The premium product from dry-milling is flaking grits (used for breakfast cereals), meal, and flour. Hominy feed is the common co-product from dry-milling that is marketed as a livestock feed.

Lastly, the dry-grind corn process is designed to subject the entire corn kernel to fermentation for the purpose of fuel ethanol production; a process that emphasizes maximum yield and energy conservation. The grains go through a process of grinding and the separated starch fraction is then cooked at low and high temperatures to gelatinize the product, and then enzymes such as α-amylase and glucoamylase are added to break the starch into simple glucose units. Because the yeast can only utilize simple sugars as a substrate for fermentation, the *S. cerevisiae* is able to ferment the glucose product into ethanol, which only takes 6-10 h. The fermented liquor is distilled, leaving behind a product called "stillage," which can be separated by centrifugation into two parts: distiller's grains and liquor (thin stillage with solubles). Unlike the processes described above, in the dry-grind corn process, the products that remain are considered more as by-products than as co-products due to the variability and inconsistency in nutrient

concentration and quality. The by-products often marketed as livestock feeds can either be sold separately or recombined, and can be offered either wet or dry. These products are known as wet distiller's grains (**WDG**) vs. dried distiller's grains (**DDG**), condensed distiller's solubles (**CDS**) vs. dried distiller's solubles, and wet distiller's grains with soluble (**WDGS**) vs. dried distiller's grains with solubles (**DDGS**; Loy, 2007). The main difference between wet- and dry-milling is that the entire grain is milled and not fractioned before the water and enzymes are added in dry milling, rather than the partitioning common of wet milling.

Sulfur Content in Distiller's Co-Products. While distiller's co-products are an appealing feedstuff from a protein and energy stand-point, they can also be markedly higher (and often variable) in S compared to other feedstuffs intended for beef cattle consumption. Whereas the S content of dry corn grain is only 0.12 %, the S content of DDGS is variable, listed at 0.33 and 0.40 % (DM-basis) in the 1984 and 1996 Beef NRC's, respectively. This variability noted in the two NRC publications reflects discrepancies and variability reported by researchers. Early reports by Carpenter (1970) and Cromwell et al. (1993) indicate tremendous variability in the nutritional characteristics in distiller's grains between sources and between batches from the same source. More recently, Spiehs et al. (2002) reported that the S concentration from 118 samples of DDGS collected from 10 ethanol plants in the Minnesota-South Dakota region varied between 0.33 and 0.74 %, on a DM-basis. Similarly, Holt and Pritchard (2004) sampled 4 ethanol plants and found that S content ranged from 0.35 to 0.69 % for DDGS; 0.36 to 0.39 % for WDG; and from 0.25 to 1.15 % for CDS. Authors from these studies cautioned nutritionists of this variation and concentration of S that exists in distiller's

grains, which cannot only influence the nutritional and physical characteristics of the product, but also be detrimental to the health of the animals fed the co-products.

The explanation for the variability in S concentrations between distiller's co-products and the grains used for ethanol production is the use of dilute sulfuric acid (H_2SO_4) at ethanol plants. Sugar-containing "wort" (mash) is sterilized with the H_2SO_4 as a measure to control pH and prevent bacterial contamination of lactic acid bacteria, thereby maintaining the integrity of the yeast used for fermentation (Hynes et al., 1997). For example, lactobacilli can grow under the conditions that are ideal for the yeasts and will compete for available substrate, therefore inhibiting yeast growth and ethanol yields. Washing the wort with H_2SO_4 allows for the yeast to be reused in subsequent batches (3-4 times / d for up to 200 d). Additionally, virginiamycin, an antibiotic produced by *Streptomyces virginiae,* has been shown to prevent or reduce potential yield losses of up to 11 % of the ethanol produced due to the growth and metabolism of lactobacilli (Hynes et al., 1997). Because the growth of contaminating lactobacilli is a major problem in industrial alcohol fermentations, both H_2SO_4 and virginiamycin are widely used by ethanol plants (Serra et al., 1980). However, while sulfur-based compounds are essential in the ruminant diet for the purpose of microbial *de novo* sulfur-amino acid synthesis, high levels of dietary S can be problematic. For example, high levels of dietary S can decrease the availability of other minerals and elicit mineral deficiencies (Goodrich and Tillman 1966; Suttle, 1974). It is critical that the high concentration of S common in distiller's grains be closely evaluated and monitored when being offered to beef cattle.

Effect of Sulfur on Copper Status. One of the most sensitive mineral interactions involves the antagonism of Cu due to excess S (Goodrich and Tillman 1966; Suttle 1974;

Kandylis 1983). Suttle (1974) demonstrated that the addition of S to the diet in either an organic or inorganic form decreased plasma Cu concentrations in sheep. Previously, work conducted by Goodrich and Tillman (1966) indicated that additional S in the diet of sheep significantly reduced hepatic Cu concentrations. In a review, Kandylis (1983) described that large quantities of dietary S intake can decrease feed intake, reduce performance, and decrease cellulose digestion in ruminants. Kandylis also described that excessive quantities (> 0.3 to 0.4 %) of S in the diet has been shown to cause toxic responses in ruminants, where generation of large quantities of hydrogen sulfide gas (H_2S) can depress rumen motility and cause thiamine deficiency (Edwin et al., 1968), and then when eructated, lead to severe distress of the nervous system, known as polioencephalomalacia (**PEM**; Gould, 1998). While Zinn et al. (1997) determined that S in excess of 0.20 % of dietary DM would lead to detrimental effects on growth performance in feedlot cattle, the NRC (1980) indicates that the maximum tolerable level of dietary S is 0.40 % (DM), which is in agreement with the values that Kandylis (1983) indicated. The S requirement of mature cows in gestation or lactation is 0.15 % of dietary DM (NRC, 1996).

Diagnostics employed for the assessment Cu status in beef cattle include (but are not limited to) analysis of hepatic tissue stores, circulating plasma or ceruloplasmin levels, and activity of the erythrocyte enzyme (CuZn) superoxide dismutase (Mills, 1987). More recently, Legleiter and Spears (2007) reported that assessment of plasma diamine oxidase, a Cu-containing enzyme responsible for the oxidative deamination of diamines (cadaverine and putrescine), their derivatives, and histamine, may serve as an effective tool to diagnose Cu deficiency in bovines. Copper concentrations have been

shown to increase in hepatic tissue with increasing levels of dietary Cu (Engle and Spears, 2000; Hansen et al., 2008). Once in the liver, Cu can be stored with metallothionein (a Cu storage protein, thought be involved with the regulation of Cu absorption), used for ceruloplasmin synthesis (required for Fe transport), partitioned as a co-factor for other enzymes, or excreted in the bile. While the Cu status of beef cattle varies with age and level of productivity, the normal range for hepatic Cu concentration in mature cows is 50 - 400 ppm (Mills, 1987). It could be elucidated from those values that liver Cu concentrations < 50 ppm would be an indication of Cu deficiency in beef cattle. A deficiency in Cu can lead to a loss of pigmentation to hair and skin, impaired fertility, diarrhea, anemia, cardiac failure, and severe distress to bones, lungs, and the central nervous system (Mills, 1987).

Nitrogen Utilization. In addition to the increased concentration of S, other nutrients become more highly concentrated in distiller's grains compared to corn. Starch, comprising nearly two-thirds of the corn kernel, is the fraction removed during ethanol production, leaving behind a by-product that is 3-fold more concentrated than corn in protein, fat, fiber and P. Klopfenstein et al. (2008) previously outlined these nutrient changes: 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. Additionally, pretreatment of the cereal grains (or other cellulosic biomass) with ammonia aids in the efficiency of ethanol production (Ko et al., 2009), which also may contribute to elevated N levels in the by-products. These increases in protein and energy make distiller's grains an attractive feedstuff for livestock, especially when the product is priced competitively among alternative protein and energy commodities. Additionally, zein, the primary type of protein found in the

endosperm of corn (Shewry and Tatham, 1990), is more resistant to degradation by rumen microorganisms than is soybean meal or casein (McDonald, 1954; Little et al., 1968). Because zein is the primary protein of corn, it is coincidentally at an elevated concentration in DDGS. In a review by Loy (2007), it is estimated that distiller's grains are about 50 % degraded by the rumen bacteria, so it could be elucidated that the remaining 50 % would be available for degradation and absorption by the animal in the orginal form. Therefore, incorporating distiller's grains could allow for a lower protein diet to meet the needs of the animal when compared to other protein sources with higher DIP (but only if the amino acid profile is sufficient). For this reason, DDGS are used extensively as a source of "escape" protein for ruminants.

In the ruminant animal exists a complex metabolic system for protein degradation and nitrogen (**N**) utilization for the purpose of *de novo* protein synthesis. The basic concepts of protein metabolism have been previously reviewed (Huntington and Archibeque, 1999; Klopfenstein, 1996) and will be relied upon for this discussion. Dietary N may be provided in many forms: nucleic acids, amino acids, proteins, peptides, amines, nitrates, nitrites, urea, and NH_3. Furthermore, endogenous sources of N can also be utilized which can become available through exfoliated GI epithelial cells, rumen microbial cell turn-over, and from urea recycling, which is synthesized by the liver in the ornithine cycle (Krebs and Henseleit, 1932). Urea from the ornithine cycle can be "recycled," and can reenter the rumen either by diffusion across the rumen wall or through saliva. Many of the N sources mentioned are readily soluble and will be degraded in the rumen by the microflora, however, some proteins, particularly those associated with NDF and ADF (referred to as **NDICP**, neutral detergent insoluble crude

protein and **ADICP**, acid detergent insoluble crude protein, respectively, NRC, 1996), will require further gastric denaturation, hydrolysis, and enzymatic break-down past the rumen.

Dietary protein offered to ruminants can be sub-divided into two categories, based on the rate and availability for degradation. The first being ruminally degradable intake protein (**DIP**), also referred to as the fraction of protein associated with rapidly fermentable carbohydrates. Carbohydrates and proteins can be classified according to degredation rates where fraction "A" represents rapidly-degradable, non-protein nitrogen (**NPN**), fraction "B1" is the intermediate protein fraction that is soluble in borate buffer, "B2" is slowly-degraded cell wall components that are soluble in neutral detergent, "B3" is slowly-degraded cell wall components that are not soluble in neutral detergent but is soluble in acid detergent, lastly "C" is the indigestible protein component associated with lignin, tannin-protein complexes, and Maillard products which are highly resistant to microbial and mammalian enzymes (Sniffen et al., 1992). Therefore, DIP is considered to be comprised largely of fractions A, B1, B2, and, partly, B3 (Sniffen et al., 1992). The second category of protein is referred to as ruminally undegradable intake protein (**UIP**), which can also be referred to as "bypass" or "escape" protein. The UIP is the fraction of dietary protein that either exceeds the metabolic needs of the rumen microbes, or requires further mechanical and chemical break-down, and is consequently passed to the lower tract for small intestine digestion in the host animal. Consequently, UIP is largely comprised of the B3 and C fractions of protein (Sniffen et al., 1992). It is likely that grazing ruminants at or near maintenance can obtain their N needs from MCP turn-over alone (NRC, 1996). However, supplementation of rumen UIP becomes more necessary

during growth, late gestation, and lactation (Klopfenstein, 1996). A linear increase in ADG was noted when rumen UIP was added to the diets of yearling steers (Karges et al., 1992) and to growing calves (Anderson et al., 1988; Goedeken et al., 1987), both of which were maintained on pasture.

The ecology of microorganisms that inhabit the rumen is diverse and survives due to a co-habitative symbiotic relationship with the host animal and due to a complicated network of numerous interrelationships. Largely, the ruminal microbial ecosystem is regarded to encompass two groups: microbes that ferment nonstructural carbohydrates (**NSC**) and those that ferment structural carbohydrates (**SC**). This segregation reflects differences in N utilization and substrate specificity. The bacteria that ferment NSC are often referred to as amylolytic bacteria for their unique ability to secrete exogenous α-amylase and rapidly utilize starch. Some of the common amylolytic bacteria of the normal flora are *Ruminobacter*, *Prevotella*, *Streptococcus*, *Selenomonas*, *Butyrivibrio*, *Eubacterium*, and *Clostridium* spp. (Cotta, 1988). The amylolytic bacteria use either NH_3 or peptides and amino acids as a N source, and will produce additional NH_3 (Russell et al., 1983; Russell et al., 1992). On the other hand, whereas mammals do not secrete endogenous cellulase and zylanase needed for the degradation of SC, cellulolytic bacteria in the rumen do. The predominant cellulolytic bacteria found in the rumen and hindguts of most herbivores are *Fibrobacter succinogens, Ruminococcus albus* and *Ruminococcus flavefaciens* (Williams and Strachan, 1984). Many of the cellulolytic bacteria assimilate only NH_3-N (Russell et al., 1992), thus forming a link between transformation of N sources listed above to NH_3 and fermentation of fiber.

Ammonia, a by-product of either dietary non-protein nitrogen (**NPN**; or urea) break-down or of the proteolysis of dietary protein, peptidolysis of peptides, and finally the deamination of amino acids can be absorbed, or diffused, across all sections of the digestive tract of ruminants. In the more acidic environment of the GI tract, NH_3 is typically protonated and is predominantly in its ammonium ion ($\mathbf{NH_4^+}$) form. Therefore, before diffusion can occur, the NH_4^+ must be converted to NH_3 at the gut wall for transport through epithelial cells, and then is re-protonated to NH_4^+ for transport into the portal system. Ammonia is removed from portal blood by the liver and is used to form urea ($\mathbf{(NH_2)_2CO}$) in a cyclic pathway, known simply as the urea cycle (Krebs and Henseleit, 1932). If N is in excess of metabolic needs, NH_3 can be returned to the GI tract through saliva or is excreted through the urinary system as $(NH_2)_2CO$.

Nitrogen Excretion. Nitrogen losses from animal agriculture to air and water are considered to be a major global environmental concern (NRC, 1996; EPA, 2006). Of all the NH_3 and nitrous oxide ($\mathbf{N_2O}$) released into the environment because of human activity, approximately 70 and 30%, respectively, are estimated to arise from livestock farming (van Aardenne et al., 2001). The IPCC (2001) agrees that agriculture and livestock farming are definite contributors to the annual increase in greenhouse gas pollution. Consequently, societal, environmental, and regulatory concerns are putting pressure on agriculture to improve methods to reduce air, water, and soil contamination.

Inefficient N utilization, due to an inadequate amino acid profile, necessitates feeding larger amounts of supplemental protein; this in-turn increases production costs and further contributes to environmental N pollution. On dairy farms, 20 to 30 % of the N consumed by cows is used toward milk protein production and muscle growth and

repair; the remainder is excreted in manure (Kohn et al., 1997). Pasture-fed ruminants have a N use efficiency of less than 10 % (Hutchings et al., 1996) as do finishing beef in a feedlot scenario (Bierman et al., 1999). In poultry or swine production, where the CP needs of the animals can be more closely met, this efficiency may average 30 to 35 % and may approach 40 % (Han et al., 2001). The N excreted in urine can be rapidly converted to NH_4^+ by hydrolysis of $(NH_2)_2CO$ due to the urease enzymes present in animal feces (Oenema et al., 2001). This effect is exacerbated in a confined animal feeding operation due to comingling of urine and feces. In the presense of microbial-derived urease, manure NH_4^+ is readily deprotonated or volatilized into NH_3 and can be lost to the air. In the atmosphere, NH_3 can be converted to aerosols which contribute to smog formation and are detrimental to human health. Nitrogen that is volatilized into the air as NH_3 returns to the surface through rainfall, which then leaches into ground water, rivers, and streams (Kohn et al., 1997).

Assessing Nitrogen Status. Distiller's grains are typically high, but variable, in protein content (ranging from 25 – 35 % CP on a DM-basis; Wheals et al., 1999) and are ~ 50 % UIP (Loy, 2007; Kleinschmit et al., 2006; Krishnamoorthy et al., 1982). This uniform combination of DIP and UIP in DDGS makes it an appealing protein source, able to advocate the N needs of both microbes and the host animal (Chalupa 1975; Waller et al. 1980; Klopfenstein 1996; Huntington and Archibeque 1999). However, it has been previously stated that because of the high concentration of protein inherent in distiller's grains, ruminant diets supplemented with the co-product (at a high % of DM) could contain excess protein, resulting in a waste of resources (Rausch and Belyea, 2005). However, early work conducted by Horn and Beeson (1969) showed that feeding DDGS

(at 5% of the diet in place of cracked corn) improved N retention and numerically decreased BUN in steers. Similarly, lambs fed either corn or milo DDG or DDGS at varying inclusion levels (ranging from 33 to 67 %, DM-basis) in urea-based diets had numerically higher g/d of N intake, but numerically decreased g/d of urinary N excretion (Waller et al., 1980). These studies suggest that feeding distiller's grains improves N status and reduced N losses to the environment. Conversely, when DDGS are fed at a high % of DM, there are alterations in ruminal fermentation and may inhibit digestion. It has been previously indicated that DDGS should not be fed above ~3 kg cow^{-1} d^{-1} (DM) which may cause a reduction in forage digestion (Klopfenstein et al. 2008) and may decrease the amount of time that animals maintained on pasture spend grazing (Caton and Dhuyvetter 1997). Nevertheless, it seems likely that N status and efficiency can be improved and excretion of N into the environment can be limited when the DIP and UIP supplied by distiller's grains are accounted for and well-managed in ruminant diets.

The ability to estimate the concentration of circulating N in animals is an important tool for determining bioavailability of dietary protein and thereby allows us to fine-tune diets so that N losses to the environment can be reduced. Useful indicators that have been employed to determine whole animal N status consist of milk (**MUN**), plasma (**PUN**), serum (**SUN**) , whole blood (**BUN**) and urinary (urea; **UN**) N excretion (Kohn et al., 2005). Changes in any of these biological indicators could be used diagnostically for determination of excess or deficient amounts of N supplied by dietary sources of CP. An increase in any of these indicators would reflect excess dietary CP provided to the animal. For example, Kohn et al. (2005) indicated that BUN is highly correlated with urine N excretion rate. Johnson and Preston (1995) suggested that PUN values greater

than 5 to 8 mg/100 mL (1.66 to 2.66 m*M*) were indicative of excessive N intake and N wastage in steers. However, Cole et al. (2003) indicated that PUN concentrations would need to be greater than 8 mg/100 mL (2.66 m*M*) to be indicative of excessive N intake and N wastage (as it equated to increased urine N) in finishing beef steers. In addition to N losses to the environment, excess N can equate to decreased animal performance. For example, Ferguson et al. (1988, 1993) indicated that a SUN concentration greater than 6.66 m*M* (20 mg/dL) is associated with reduced conception rates in lactating dairy cows. In earlier work, Fenderson and Bergen (1976) demonstrated that increasing the dietary CP (from 10 to 40 % using soybean meal and isolated soy protein concentrates) had a marked (although not statistically significant) effect on DMI in growing Holstein steer calves, where the high CP rations depressed intake on d 2 and 3 followed by a recovery to initial intake levels between days 5 to 10 of a 14-d feeding period. More recently, Kane et al. (2004) suggested that increasing the amount of dietary CP (more specifically, amounts of UIP) supplemented in the diets of breeding beef females negatively affected pituitary and ovarian function, and thereby negatively influenced reproductive performance.

In contrast, decreased milk, blood, or urinary N would be a reflection of insufficient dietary CP provided to meet the needs of productive or anabolic growth needs of growing or breeding animals. Inadequate dietary intake of CP results in a catabolic state or the mobilization of fat and glycogen stores (due to increased levels of catabolic hormones such as glucagon, glucocorticoids, and catecholamines) and 'muscle wasting' (also referred to as protein degredation; Castaneda, 2002). Muscle wasting is characterized by increased muscle protein catabolism compared to muscle protein

accretion, whereby skeletal muscle is degraded to maintain proper N balance (Castaneda, 2002). Therefore, changes in these biological markers would not only be used as indicators of dietary CP excesses and deficiencies, but could be used to assess whole animal N status. Thus, changes in milk, blood, or urinary N have been widely used by the animal science community as a simple and reliable approach for the assessment of N status and losses of N to the environment (Kohn et al., 2005).

Feeding DDGS to Breeding Females. Breeding beef females managed under range conditions typically have grazing access to low-quality forages (< 6 % CP, DM basis) from late summer through winter (Clanton and Zimmerman, 1970), until early spring growth of new pasture. Supplemental protein offered to ruminants consuming medium- to low-quality forages is an effective method to maintain or increase BW and BCS (Clanton and Zimmerman, 1970; Owens et al. 1991; Rusche et al., 1993; Bohnert et al. 2002; Schauer et al. 2005; Archibeque et al. 2008). While DDGS contain up to 60 % CP as UIP, they also contain 8 to 12 % fat (DM-basis; NRC, 1996). Therefore, it seems intuitive that supplementing the diets of breeding beef females with distiller's grains, particularly those maintained on low-quality forages, would benefit not only from the additional rumen DIP, but also from the additional energy. Recently, this hypothesis was tested. MacDonald et al. (2007) conducted a study on heifers grazing smooth brome grass pastures to determine the relative contributions of UIP and dietary fat on performance (DMI and ADG). As expected, supplementing with DDGS increased ADG, but surprisingly decreased forage intake. The authors theorized that the decrease in forage intake may have been due to satiety brought on by the additional intake of fat, and that the supplement met the energy needs of the heifers without additional intake.

Similarly, Schauer et al. (2005) reported that providing supplemental protein to cows grazing low-quality forage increased BW and BCS gain, but decreasing grazing time. It could be concluded that an increase in performance of the DDG-supplemented heifers could not be independently explained by either the intake of rumen UIP or fat, but rather the combination of both (MacDonald et al., 2007).

In beef cows, protein deficiencies have been shown to affect reproduction negatively (Nolan et al., 1988; Sasser et al., 1988). Feeding distiller's grains to beef cows has been shown to improve conception and pregnancy rates (Martin et al., 2007). This effect of supplemental fat and rumen UIP on reproductive performance has been well established (Wiltbank et al., 1962; Bond and Wiltbank, 1970; Short et al., 1990). Most recently, Engel et al. (2008) offered DDGS in the prepartum diets of 2 yo primiparous beef heifers and found that supplementing with DDGS improved BW gain during late gestation and benefited pregnancy rates following calving. The authors discussed that while the UIP provides amino acids needed for protein metabolism and fetal calf growth, the fat supplementation likely influenced follicular growth and affected the levels of circulating metabolic hormones, such as growth hormone (**GH**) and insulin. Because DDGS contain both supplemental fat and rumen UIP, it is a feedstuff that cow-calf producers should consider for the optimization of BW, ADG, BCS, and reproductive performance in their breeding females.

Feeding DDGS to Growing and Finishing Cattle. In early reviews by Chalupa (1975), Mercer and Annison (1976), and Kempton et al. (1977) the necessity of offering rumen UIP (referred to commonly in those papers as "by-pass" protein) for maximized production and feed efficiency of growing ruminants was discussed. Later, Waller et al.

(1980) proposed that feeding either DDG or DDGS as a source of rumen UIP would provide an ideal opportunity for urea to be offered as a source of DIP. The authors conducted 3 experiments. First, Exp. 1 demonstrated that lambs had higher DM digestibility when either DDG or DDGS was added to urea-supplemented diets (the diets were based on ground corn cobs and cane molasses). Then, in Exp. 2 and 3, growing steers fed urea-based diets supplemented with DDG or DDGS had higher ADG and substantially improved F:G than steers fed only urea. In agreement, Firkins et al. (1985) demonstrated that supplementing the diets of growing feedlot steers dramatically improved ADG and F:G when compared to controls. The authors also reported that steers fed DDG had the slowest rate of *in situ* DM disappearance and the highest % of DM remaining after 36 hours between different protein supplements. The authors conceded that these results were partly due to the physical nature of the test articles (i.e. DDGS, WDGS, DCGF, WCGF), rather than true digestibility. It could be speculated that the authors were implying that there were differences in solubility or particle size of the test articles, which may explain the differences seen in DM disappearance. It also seems likely that of the protein supplements offered, that the DDG had the highest fraction of non-soluble protein and required the greatest gut transit time.

More recently, feeding distiller's grains in the rations of finishing beef cattle has increased ADG and efficiency of gain compared to rations without it (Ham et al., 1994; Al-Suwaiegh et al., 2002). It seems likely that this additional performance could be attributed to the increase in NE_G content of rations containing distiller's grains; particularly when rations are balanced for DM and CP intake. However, there appears to be a limitation to the level of distiller's that should be fed to growing and finishing cattle.

For example, Depenbusch et al., (2009) offered increasing levels of DDGS in the diets of finishing beef heifers (inclusion levels tested were 0, 15, 30, 45, 60, and 75 %, DM-basis) and reported that DMI, ADG, and final BW decreased at each level of DGS fed above 15 % (DM-basis), indicating that 15 % inclusion was optimal. However, in that study, marbling scores and USDA quality (Choice and Prime) yield grades were not affected by the level of DDGS fed. In a similar study design, Leupp et al., (2009) offered increasing levels of DDGS in the diets of heavy-weight ruminally and duodenally cannulated beef steers (inclusion levels tested were 0, 15, 30, 45, and 60 %, DM-basis) and indicated that replacing dry-rolled corn with up to 60 % DDGS in 70 % concentrate diets resulted in no adverse effects on total tract OM digestion (although OM intake was reduced at 60 % DDGS inclusion). However, the authors reported that while total m*M* concentration of VFA decreased at each level of DDGS fed above 15 % (DM-basis), acetate concentrations decreased as propionate concentrations increased at each level. In the Leupp study, the authors concluded that the optimal inclusion of DDGS was higher than what Depenbush had indicated, suggesting that 45 % inclusion in the diets of growing beef steers would maximize digestion and fermentation. However, performance and carcass data were not collected in the Leupp study, so it is difficult to reconcile with this conclusion. Vasconcelos and Galyean (2007) reported that 82.76 % of beef nutritionists are currently formulating for the inclusion of 5 – 50 % ethanol co-products in beef finishing rations (average = 16.5 %; mode = 20 %) on a DM-basis. It could be elucidated that the limitations in amounts of distiller's grains that could be fed is likely due to the excess levels of, or imbalance of, nutrients such as fat, P, and S (as has been previously discussed).

E. coli O157:H7

Recent research indicates that there is a positive association between feeding distiller's grains (or brewer's grains, a fermentative co-product from the brewing industry similar to distiller's grains) and the prevalence of the pathogen *Escherichia coli* O157 in feedlot cattle (Dewell et al., 2005; Jacob et al., 2008a; 2008b; 2008c). With approximately 83 % of beef nutritionists reporting that they are currently formulating beef finishing diets to include distiller's co-products (Vasconcelos and Galyean, 2007), these findings have important ramifications for food safety. The GI tracts of beef cattle are recognized as a reservoir for the food borne pathogen, shiga toxin-producing *E. coli* O157:H7, which has been implicated with human death, hospitalizations, and disease (Callaway et al., 2003). It has been approximated that human illnesses caused by food-borne pathogens cost the U.S. economy $6.9 billion and result in 1,600 deaths each year (ERS/USDA, 2001). Beef carcasses can become tainted with pathogens during harvest due to direct contact with infected fecal matter (Elder et al., 2000).

In a review prepared by Rasmussen and Casey (2001), it was stated that *E. coli* O157:H7 prevalence is widespread among U.S. cattle herds and individual animal prevalence is low and transient. In that review, it was summarized that among multiple variables such as environmental, feed, water trough load, insects, weather patterns during summer and fall months, manure handling, and stress, that diet composition is also likely a strong influence on the survival and establishment of *E. coli* 0157:H7 in the GI tract of beef cattle. In a series of studies Jacob et al. (2008a; 2008b; 2008c) reported that distiller's grains in the diets of cattle caused increased fecal prevalence of the pathogen.

First, the 2008a study indicated that when Holstein bull calves were orally inoculated with *E. coli* O157 that the pathogen persisted in the gut and feces when distiller's grains were fed. The 2008b trial demonstrated that 25 % inclusion of DDGS in finishing ration of finishing heifers, increased the fecal prevalence of *E. coli* O157 compared to cattle not fed distiller's grains. Similarly, the 2008c study indicated increased prevalence of *E. coli* O157 in the feces of cross-bred yearling heifers fed 25% WDG with solubles in finishing diets. In agreement with the Jacob et al. trials, Dewell et al. (2005) demonstrated in a multistate epidemiologic study, that *E. coli* O157-positive fecal samples were 6 times higher in cattle fed brewer's grains. In contrast to these reports, Jacob et al. (2009) most recently reported no effect of diet on prevalence of the organism when 25 % distiller's grains were fed to finishing cattle. The authors of the 2009 study concluded that a combination of low prevalence of the pathogen and variability of nutrient content in the distiller's grain may have attributed to the disparity seen between the 2009 trial and their earlier experiments.

Mode of Action. It has been hypothesized that the increased prevalence of *E. coli* O157 associated with feeding distiller's grains or brewer's grains is possibly due to changes in the micro flora environment of the hind gut, which is the primary colonization site of the pathogen (Grauke et al., 2002; Jacob et al., 2008c). Firkins et al. (1985) demonstrated that feeding 35 % inclusion of a protein- and fat-dense co-products in place of starch-based ingredients such as corn in the diet results in an elevated mean ruminal pH (at 9 and 12 h), post-feeding. However, when the same co-products were offered at a higher inclusion rate (70 %), the effects on ruminal pH were reversed. It seems likely that the combination of high concentrations of RUP, fiber, and fat components in

distiller's grains in the absence of starch would shift digestion from rumen to hindgut fermentation and that the presentation of these dietary constituents in the hindgut could promote hind gut proliferation of *E. coli* O157:H7. Van Baale et al. (2004) demonstrated that cattle fed forage diets were *E. coli* O157-positive longer and with higher numbers than cattle fed grain-based diets. The authors of that study also reported that *E. coli* O157:H7 was detected in cecal and colonic digesta, but not from the rumen, confirming that the pathogen colonizes the hind gut (and not the rumen) of ruminants. The reasons that *E. coli* O157:H7 has preference for colonizing the hindgut ecology over the rumen has not been elucidated. It seems logical that the environment and pH, in addition to reduced competition for attachment sites and available substrate, is more conducive for survival of the pathogen in the hindgut as compared to the rumen. Additionally, it has been reported that *E. coli* O157:H7-positive fecal pats and droppings (from cattle and wildlife) have been recovered from the environment at low levels ($\leq$ 1.01 %; Renter et al., 2003).

As previously described in a microbiological review (Griffin and Tauxe, 1991), *E. coli* O157:H7 is a gram-negative bacillus, where the "O" refers to the somatic (cells forming the body of an organism), and the "H" to the flagellar antigens. This serotype does not ferment sorbitol rapidly (hence why sorbitol MacConkey agar is used for rapid screening), produce β-glucuronidase (enzymes that breakdown complex carbohydrates), nor grow well above 41 °C (unlike most *E. coli* prototypes). The virulence properties of *E. coli* O157:H7 is not due to invasion or intracellular multiplication, but rather to adherence to intestinal mucosal cells and the production of Shiga-like toxins I and II (a toxin originally isolated from *Shigella dysenteriae*). The adherence of the pathogen

produces a distinct microscopic lesion due to intimate attachment on the mucosa of the colon. Shiga-like toxins are cytotoxins that kill Vero (kidney epithelial cells) and HeLa (immortal cervical cancer cells) cells *in vitro* by binding to cellular receptors, becoming internalized, and leading to the subsequent destruction of ribosomal RNA, thereby ceasing protein synthesis of the cells. Infection by Shiga-like toxin-producing *E. coli* has been associated with hemorrhagic colitis (bloody diarrhea) in humans.

Benefits of Feeding Ionophores

Mode of Action. Currently, it is conventional practice among U.S. beef feedlots to implement growth promotants such as ionophores or estrogenic hormone implants or both, in grow-finish beef programs to optimize lean muscle growth and yield of beef carcasses. The basic cellular mode of action is well documented and has been reviewed (Pressman, 1976; Schelling, 1984). Briefly, the term "ionophore" relates to the capacity of a molecule to transport ions across a biological membrane. Ionophores form lipid-soluble complexes with polar cations such as K^+, Na^+, Ca^{2+}, and Mg^{2+} and promote the transfer of these cations from an aqueous environment across the phospholipid bi-layer of biological membranes. This transport mechanism increases the ionic permeability of the cations. The ionophore is capable of attracting cations due to an inner ring structure rich in oxygen atoms, creating a polarized ion-dipole space, allowing for a highly compatible interaction between the molecules. The interior of the ionophore-cation complex is unstable in an aqueous environment, but once the complex interfaces within the lipid membranes, it becomes more stable and solublizes in the lipid environment; hence the reference to "lipid-soluble complexes." In short, ionophores shield the electric charge of

ions by providing a polar environment for the ion, therefore allowing passage through membranes.

Prevention of Coccidiosis. At least 70 ionophores have been identified, with some being natural products isolated from microorganisms and others synthetically designed for specific applications. Monensin (**MON**), most widely known on a commercial-basis as Rumensin® (Elanco, Division of Eli Lilly and Company, Greenfield, IN), is a commonly used ionophore in U.S. beef production systems. Monensin is a biologically active compound produced by *Streptomyces cinnamonensis* and is used widely as an anticoccidial drug (Fitzgerald and Mansfield, 1973; Haney and Hoehn, 1967). Coccidiosis, most prevalent in young cattle, costs the beef cattle industry at least $100 million annually (Schelling, 1984). The oocysts of protozoan parasites, such as *Eimeria* spp. (species that cattle are most sensitive to), are shed in the feces of affected animals and transmitted from animal to animal by the fecal–oral route (Merck, 2005). Preventing the transmission of the parasites through the therapeutic application of MON improves animal health, decreases veterinary expenses, and increases DMI and growth performance in growing beef animals (Goodrich et al., 1984).

Effect of Ionophores on Growth Promotion. In addition to the prevention of coccidiosis, the prophylactic use of MON has been shown to improve the performance of growing and finishing beef animals. While the mechanism by which ionophores enhance growth has not been fully elucidated, it is generally believed that the action can largely be attributed to alteration of the composition of the animals' natural gastro intestinal micro flora and reduced competition for establishment of bacterial population growth and access to substrate (Fuller et al., 1960). First, by decreasing the number and presence of

pathogenic bacteria in the GI tract of animals, the natural flora of the GI tract is better able to utilize feedstuffs which equates to increased ADG and growth performance of the animal. Additionally, MON has shown to alter the VFA profile in the rumen, which likely attributes to enhanced growth performance. By manipulating the mobility of ions across biological membranes, MON can increase the molar proportion of propionate at the expense of lactate and concurrently decrease the molar proportions of acetate and butyrate produced in the rumen (Chalupa, 1977). *In vivo* and *in vitro* studies using MON demonstrated increased propionate yield at the expense of acetate and butyrate when applied in ruminant systems (Richardson et al., 1976). This shift toward propionate is more energetically efficient for the ruminant animal because propionate is a precursor to gluconeogenesis and because propionate synthesis is accompanied by a reduction in the amount of CH_4 produced in the rumen (Hobson and Stewart, 1997). Bergen and Bates (1984) associated increased growth performance with reduced production of methane, which could also result in increased efficiency of energy metabolism in the rumen. Richardson et al. (1976) speculated that the re-distribution of VFA production resulted in a 5.6 % increase in gross energy and contributed to an increase in feed efficiency. In a review of 228 trials, Goodrich et al. (1984) condensed and summated that growing feedlot cattle receiving MON had increased daily weight gain, DM digestibility, and feed efficiency in addition to decreased feed intake, lactic acid production, and incidence of coccidiosis compared to control animals.

These changes in ruminal fermentation have been shown to "neutralize" and "stabilize" the rumen environment, thus preventing the incidence of acidosis and bloat when cattle are fed a diet rich in carbohydrates (Nagaraja et al., 1981). Therefore, MON

is most often fed in diets high in rapidly fermentable carbohydrates as a preventative measure against digestive disturbances. For instance, feeding MON has been shown to alter the microflora of the rumen, specifically acting as an antibiotic against Gram-positive, lactate-producing bacteria such as *Streptococcus bovis* and *Lactobacillus* spp. (Dennis et al., 1981; Chow et al., 1994), both of which have been implicated with the initiation of lactic acidosis (Owens et al., 1998). When lactate (and other organic acids) accumulates in the rumen it causes a depression in rumen pH; these biochemical changes create micro lesions in the rumen wall, allowing increased permeability of particulate matter, that would not otherwise normally be able to freely enter portal circulation. This influx of particulate matter, including bacteria, accumulates in hepatic tissue causing inflammation and infection, which can lead to liver abscesses. Due to the neutralizing properties of MON, the ionophore has been shown to reduce the incidence of liver abscesses (Nagaraja and Chengappa, 1998). For the sake of brevity, acidosis will not be further discussed here, so the reader is directed to a review from ruminant literature (Owens et al., 1998).

Effect of Ionophores on Fecal Prevalence of E. coli O157. Fecal shedding of *E. coli* O157:H7 is indicative of the ability of the organism to persist or colonize the GI tract of beef cattle (Van Baale et al., 2004). Previous research employing ionophores for the reduction of the organism in the feces of ruminants indicates that there may be an opposite effect (Dennis et al., 1981; Henderson et al., 1981; Schelling, 1984). It is hypothesized that because ionophores inhibit the growth of Gram-positive, lactate-producing rumen bacteria, that feeding ionophores to ruminants would increase the incidence of *E. coli* due to lack of competition in the hind gut. In an epidemiological

evaluation of 36 dairy herds, Herriott et al. (1998) reported a positive association between the prevalence of the pathogen and the inclusion of ionophores in the feed of dairy cattle. The association indicated that the prevalence of Shiga toxin-producing *E. coli* was higher in cattle fed MON compared to those that were not (1.75 versus 0.69 %, respectively). Conversely, Van Baale et al. (2004) demonstrated that ionophore supplementation decreased the duration of *E. coli* O157:H7 prevalence in the feces of experimentally-inoculated forage-fed cattle. Additionally, the authors reported no effect of ionophore treatment on the prevalence of the pathogen in the rumen fluid of those same animals. Edrington et al. (2003) found no effect of ionophore treatment on the fecal shedding of *E. coli* O157:H7 in experimentally infected lambs. These differences and inconsistencies between studies lends to inconclusivity on the effectiveness of ionophores on the persistence of the pathogen.

Due to recent public scrutiny that the prophylactic use of ionophores as growth promoters in beef cattle may contribute to antibiotic resistance (PCIFAP, 2006), it seems likely that the use of ionophores will be under greater surveillance or completely prohibited in the future. This public perception exists despite that ionophores are unlikely to contribute to antibiotic resistance (Russell and Houlihan, 2003). Aside from application of antibiotics to beef cattle, Spellberg et al. (2008) indicates that there are multiple contributors to the emergence of multidrug-resistant microbes, which include (but are not limited to): physician misuse and mis-prescription of antibiotics, patients self-prescribing their own duration and dosage of prescribed antibiotics, and that microbes are the most numerous, robust, diverse, and adaptable organism on the planet. Additionally, because antibiotics are naturally-occurring phenomena that humans did not

invent, microbes do not need ionophores to create antibiotic resistance (Spellberg et al., 2008). Regardless, exploration of natural strategies that are effective at reducing food-borne pathogen load at the preharvest stage is needed.

Yeast Culture as a Natural Alternative for Naturally-Raised Beef. There is growing consumer demand for natural and organically-grown beef (Grannis and Thilmany, 2000; Thompson et al., 2007). The USDA (2009) designates that naturally-raised animals are to be grown without the use of growth hormones or antimicrobials. Under this designation, while ionophores are exempt when administered for the purpose of coccidiosis control, they are otherwise prohibited in naturally-raised beef programs. Therefore, establishing an economically competitive, natural alternative to ionophores, that fits under the USDA's designation for natural and organically-grown beef, and that does not compromise end-product quality, is of interest to researchers and beef producers. Such an alternative that could be widely accepted in the animal industry is YC.

While no published literature was available on the effects of YC on *E. coli* prevalence at the time of writing this review, there has been extensive work done with the application of bacterial DFM in beef cattle. It has been hypothesized that feeding DFM might either directly or indirectly promote the growth of natural flora bacteria that would compete with, or be antagonistic to, pathogenic bacteria (Callaway et al., 2003). Nurmi et al. (1992) suggested that competitive exclusion or displacement of pathogenic bacterial populations is the likely mode of action of bacterial DFM; establishing a competitive natural flora would eliminate both gut epithelial attachment sites and available substrate for invading food-borne pathogens. For example, some strains of lactobacilli express antimicrobial action against the pathogen through the release of toxins (Gilliland and

Speck, 1977; Reiter and Harnulv, 1984). Brashears et al. (2003) and Elam et al. (2003) reported that dietary administration of 1 x 10^9 CFU of *Lactobacillus acidophilus* decreased (but did not eliminate) both the shedding of *E. coli* O157:H7 in the feces of beef steers and the number of *E. coli* O157:H7-positive hides at harvest without detrimental effects on growth performance. In the Brashears study, the pathogen was 49 % less likely to be detected in the *Lactobacillus*-fed steers than those not receiving the DFM treatment. Similarly, Stephens et al. (2007) reported that feeding a lower dosage (1 x 10^7 CFU) of *L. acidophilus* than what was used in the other studies caused *E. coli* O157 to be 74% less likely to be isolated from the hides of cattle than those not receiving a DFM treatment.

In conclusion, while potential applications of yeast technology are expanding rapidly in animal nutrition, our understanding of how the application of yeasts can fully benefit the beef industry has not been fully elucidated. Our understanding of how YC and the fermenatative by-products of yeast from the ethanol industry can most benefit the beef industry is limited. However, it seems reasonable that expanding our knowledge in this area could render benefit to the beef industry.

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CHAPTER II

FEEDING DRIED DISTILLERS GRAINS IN LIEU OF STANDARD RANGE CUBES TO PREGNANT BEEF COWS MAINTAINED ON LOW-QUALITY FORAGE IMPROVED ECONOMIC RETURNS WITH LIMITED IMPACTS ON SERUM UREA NITROGEN (SUN) OR TRACE MINERAL STATUS OF THE COWS OR THEIR OFFSPRING

Feeding dried distillers grains in lieu of standard range cubes to pregnant beef cows maintained on low-quality forage improved economic returns with limited impacts on serum urea nitrogen (SUN) or trace mineral status of the cows or their offspring[a,z]

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Abbreviations: ADFICP, acid detergent fiber insoluble crude protein; **CON,** control diet supplemented with sunflower meal-based range cubes; **DIP** degradable intake protein; **DDG,** diet supplemented with DDGS; **DDGS,** dried distiller's grains with solubles; **EE** ether extract; **NDFICP,** neutral detergent fiber insoluble crude protein ; **NFE,** nitrogen-free extract; **SBW,** shrunk body weight; **SUN,** serum urea nitrogen; **UIP,** undegradable intake protein.

[a] This manuscript was prepared following Canadian Journal of Animal Science style and form.

ABSTRACT: Two-hundred sixteen cross-bred, multiparious, beef cows in the last third of pregnancy were blocked by age and randomly assigned to 1 of 6 pasture replicates (n = 3) and fed 1 of 2 dietary supplements: range cubes (**CON**) or dried distiller's grains with solubles (**DDG**). Cows also had continuous access to pasture and a loose mineral and were offered hay. Supplementing with DDGS increased cow BW on d 77 ($P < 0.001$), BCS on d 28 and 54 ($P < 0.001$), and ADG and gain as a percentage of BW ($P < 0.05$) throughout the study. There was no treatment effect ($P = 0.30$) on cow SUN. The DDG-supplemented cows had increased hepatic Cu, Co, and Mn concentrations ($P \leq 0.05$), but Fe decreased ($P = 0.02$), and Zn tended to be higher ($P = 0.08$) by d 77. For calves, the only effect of treatment was calves from DDG-supplemented cows tended to have increased birth weight ($P = 0.10$). Feeding DDGS yielded a $13.86 / cow savings over the duration of the trial. Supplementing pregnant beef cows on winter range with DDGS improved cow performance, had limited impact on nitrogen or mineral status, and was more economical to feed.

Key words: beef cows, copper, distiller's grains, mineral status, serum urea nitrogen, sulfur

Rapid growth of the ethanol industry has provided an increasing inventory of corn distiller's grains available for feeding beef cattle. Distiller's grains are extensively used as a source of more highly concentrated NDF, CP, fat and P than corn on a DM basis (NRC 1996) and of rumen undegradable intake protein (**UIP**; Satter et al. 1977; Klopfenstein et al. 1982; Firkins et al. 1984). Winter range conditions in typical cow-calf

production systems do not provide adequate nutrition to support the desired productivity of pregnant, grazing animals. Supplemental protein offered to ruminants consuming medium- to low-quality forages improves body weight gain or decreases BW and BCS loss (Owens et al. 1991; Bohnert et al. 2002; Schauer et al. 2005; Archibeque et al. 2008). Therefore, dried distiller's grains with solubles (**DDGS**) may serve well as an economical CP and energy supplement for ruminants maintained on dormant winter range. However, DDGS can be markedly higher in S than other typical feedstuffs (NRC 1996). Feeding high levels of S can decrease the availability of other minerals (Goodrich and Tillman 1966; Suttle 1974) and have a negative impact on cow health and performance (Kandylis 1983). Additionally, distiller's grains can be variable in protein content (25 to 35% CP on a dry-matter basis; Wheals et al. 1999). Therefore, diets supplemented with DDGS could contain excess protein, resulting in a waste of resources and an increase in blood urea nitrogen (Rausch and Belyea 2005). However, there has been relatively little research focused on the use of distiller's grains as a supplement for pregnant, range cows consuming low- to moderate-quality forages. Thus, the objective of this study was to evaluate the potential of DDGS as a supplement for range cows during the last-trimester of pregnancy. Our hypothesis was that limit-fed DDGS will be an adequate and economical choice as a winter feed supplement to cows grazing dormant winter range, without detriment to performance, nitrogen status, or trace mineral status of the cows or their calves.

MATERIALS AND METHODS

Animals and Diets

All sampling techniques, animal use and handling were pre-approved by the Colorado State University Institutional Animal Care and Use Committee.

Eight days before the start of this experiment (January 4th, 2008), 216 cross-bred (Black Angus-based), multiparious beef cows at the Eastern Colorado Research Center (Akron, CO) were blocked by age ("young cows;" 3-year-old cows vs. "mature cows;" > 3 years-of-age), and then ranked and stratified for known sires of their calves, initial BW (mean = 575 kg, SD = 59 kg), and expected calving date (mean = March 24, 2008, SD = 15 d), and randomly assigned to one of six experimental units (n = 3/treatment). Each treatment group consisted of one replicate of young cows (n = 45) and 2 replicates of mature cows (n = 31 or 32), such that 108 cows were assigned to each treatment. The replicates were randomly assigned to treatments: a control diet supplemented with sunflower meal-based range cubes (**CON**) + a loose mineral, or a diet supplemented with DDGS (**DDG**) + a different loose mineral. Diets were formulated to meet daily TDN and CP minimum requirements for the last third of pregnancy (NRC, 1996). Unique micro / macro loose mineral supplements were formulated to complement the range cube and DDGS supplements. In designing the 2 mineral supplements, it was intended that the DDG mineral be elevated in Ca and decreased in P, but otherwise the two minerals were the same in salt and trace mineral concentrations. Range cubes and loose minerals were each custom-made for the purposes of this experiment by a commercial feed-mill (Cargill Animal Nutrition, Sterling, CO) and nutrient composition of each is shown on Tables 1 and 2. Range cubes (fed on ground) and DDGS (fed in bunks) were provided in equal meals, thrice weekly (Monday, Wednesday, and Friday), and offered at a location next to the water source in each pasture. The mineral supplement was provided ad libitum in

free-choice mineral feeders near the water source in each pasture. Range cubes were fed for a target intake of approximately 3.5 kg cow^{-1} $feeding^{-1}$ (DM), DDGS were fed for a target intake of 3.4 kg cow^{-1} $feeding^{-1}$ (DM), and each loose mineral was provided for a target intake of 110 g cow^{-1} d^{-1} (DM). Feeding levels for the range cubes and DDGS was determined based on the two supplements providing similar intakes of CP.

Intake values for DMI cow^{-1} d^{-1} were estimated and based on prediction equations (NRC, 1996). Total DMI was estimated by using the following equation: total DMI, kg = intake of NE_M (Mcal cow^{-1} d^{-1}) / average NE_M (Mcal/kg) concentration of pasture, hay, supplement, and mineral. Intake of NE_M was estimated using a prediction equation for pregnant beef cows (NRC, 1996) based on dietary energy concentration: intake of NE_M = shrunk body weight (**SBW**)$^{0.75}$ *(((0.04997 * (average NE_M^2 of feedstuffs not including pasture)) + 0.04631). The SBW was calculated using this formula: SBW = BW - (BW * 0.04). While disappearance of delivered hay, DDGS or CON supplement, and mineral in each pasture replicated was known, disappearance of pasture was not measured. Therefore, pasture DMI was arrived at by subtracting DMI of hay, DDGS or CON supplement, and mineral from the total estimated DMI value. Calculated nutrient intake of cows is reported in Table 3. Based on calculated average intake of DM, CP, crude fat, NDF, ADF, TDN, DE, ME, NE_L, NE_M, NE_G, Ca, Na, S, Cu, Zn, Co, and Mn was greater in the DDG cows compared to the same age group of cows from the CON treatment. In contrast, calculated average intake of NFE, P, Mg, K, Fe, and Mo was greater in the CON cows compared to the same age group of cows from the DDG treatment. Based off of the prediction equation for DMI, DDG cows had higher pasture DMI than CON cows (data

not shown; 7.02 vs 6.58 kg cow^{-1} d^{-1}, respectively). Calculated pasture DMI equated to 1.2 % of BW.

Treatments were fed from d 200 of gestation through calving and animals were housed by replicate in six separate pastures. All cows were maintained on native winter pastures (16 ha for young cows and 32 ha for mature cows) that consisted primarily of blue grama (*Bouteloua gracilis*), prairie sandreed (*Calamovilfa longifolia*), and needle-and-thread grass (*Stipa comata).* Replicates were rotated among pastures following each data collection day (d 28, 54, and 77) in order to minimize pasture effects. Supplemental sorghum and triticale hay were provided to compensate for poor winter forage quality and the nutrient composition is shown on Table 1. The young cows were offered 3 kg cow^{-1} d^{-1} (DM) of triticale hay throughout the study in an effort to maintain body condition, whereas mature cows were fed 5.82 kg cow^{-1} d^{-1} (DM) of sorghum hay only on 12 days of extreme winter weather conditions (when the standing range was snow and ice covered). Each replicate (within age block) was offered exactly the same amount of hay on each day of hay delivery.

The 85 d (77 d exposed to treatment + sample collection 8 d prior to onset of exposure to dietary treatment) study was conducted from December 2007 to March 2008, and calves were weaned in September 2008. Cows were vaccinated against *Bovine coronavirus* (BCV) and *Clostridium perfringens* (Scour-guard 4KC, Pfizer Animal Health, New York, NY) on d - 8 and again on d 54, and dewormed (Ivermectin pour-on 5mg / mL, IVX Animal Health, St. Joseph, MO) on d - 8. Before the start of the study, the cows were individually rectally palpated for confirmation of pregnancy and assigned an expected calving date. For more intensive management during calving,

those cows expected to calve early (between d 54 and 77) were sorted and removed from their respective treatment group on d 54, and then placed in a separate pasture. The early-calving cows were assigned a calving covariate indicative of the number of days between d 54 of the study and their calving date to account for variation in exposure to treatment. Those cows that were expected to calve later remained in their respective treatment groups on pasture. On d 77, following data collection, late-calving cows were removed from exposure to their respective treatment, placed in the calving pasture along with early cows, and then assigned a respective calving covariate. Once cows were removed from treatment and placed in the calving pasture, they were offered free-choice triticale hay + the control mineral. Exposure to dietary treatments ended for all cows by d 77. Birth and weaning weights were collected on each calf.

Sample Collection

Feedstuffs. Random samples of water, range cubes, DDGS, mineral, hay, and pastures were sampled throughout the study for nutrient analysis (Tables 1 and 2). Range cubes, mineral, and DDGS were sampled upon each new delivery. On d 21 and 42 of the trial, pasture samples were clipped at ground level from 5 random sites (each site was ~ 609 mm^2) from each pasture, water samples were collected in sterilized plastic containers from each water tank, and 2 random core samples were taken from each bale of hay. All feed samples were weighed, dried at 55°C for 48 h, and then reweighed for calculation of DM. Samples were then ground in a Wiley Mill (Thomas Model 4, Swedesboro, NJ), passed through a 1-mm screen, and then stored in a sealed, plastic bag at 4°C pending analysis.

Cow and Calf Performance. Unshrunk BW and BCS were collected on d - 8, 28, and 54 for all individuals, in addition to d 77 for late-calving cows. The BCS measurements collected were on a scale of 1 to 9 (1 = emaciated, 9 = obese; Richards et al. 1986) and were assigned by two technicians, one assigning a visual score and the other a palpation score, which were then averaged for each cow for each collection day. All calves were weighed at birth and again at weaning.

Serum Urea Nitrogen. To determine the effect treatment had on serum urea nitrogen (**SUN**), whole blood was collected via jugular venipuncture in trace-mineral free Vacutainer tubes (Becton Dickinson Co., Franklin Lake, NJ) on days -8, 28, and 54 from all individuals, plus d 77 from the late-calving cows. Once collected, whole-blood samples were placed on ice in a cooler for transportation and stored over-night at 4°C before being centrifuged at 1,378 x *g* at 4°C for 15 min. Serum was then transferred to acid-washed storage vials, capped, and stored for pending analysis at - 20°C.

Trace Mineral Status. Trace mineral status of cows was measured using collection of liver biopsy samples from a randomly selected subgroup of animals (five per replicate, n = 30) on d - 8 and 54, in addition to d 77 for the late-calving cows. However, 1 cow from this subset aborted her calf during the final trimester, so her individual data were removed, therefore the total number of animals used for the liver biopsy collection was adjusted to n = 29, for both cows and calves. Liver tissue was collected from the cows before initiation of treatment to estimate herd mineral status. Liver biopsy samples were also obtained from their calves (< 7 days-of-age). The liver biopsy method was accomplished by using the true-cut technique previously described by Pearson and Craig (1980) and as modified by Engle and Spears (2000). Following

collection, samples were immediately rinsed with deionized H_2O, placed in an acid-washed polypropylene tube, capped, and placed on ice in a cooler for transportation before storing at -20°C for pending analysis.

Sample Analysis

Feedstuffs. Range cubes, DDGS, mineral, hay, and pasture samples were analyzed for DM, CP, neutral detergent fiber insoluble crude protein (**NDFICP**) and acid detergent fiber insoluble crude protein (**ADFICP**) fractions (NRC, 1996), NDF, ADF, ether extract (**EE**), and ash using AOAC (2005) methods (CP, 968.06; NDF, 2002.04; ADF, 973.18; EE, 920.39; and ash, 942.05). Nitrogen-free extract (**NFE**) was determined by difference. For determination of Ca, P, Mg, K, Na, S, Cu, Zn, Fe, Co, Mo, and Mn concentrations, all water and feedstuff samples were shipped over-night in coolers with ice packs to a commercial lab (Michigan State University, Diagnostic Center for Population and Animal Health, Lansing, MI) for analysis. The range cubes, DDGS, hay, and pasture samples were weighed, then digested in individual sealed vessels with nitric acid at a 1 mL acid : 100 mg feed ratio and placed in an oven over-night at 95°C. The next day, 5 ppm of yttrium was added to the digested samples as an internal standard, and then the samples were diluted with Type 1 H_2O (distilled, deionized, and filtered through a 0.45 micron filter) to a final volume of 25 mL (approx. 50x dilution). From each digested and diluted sample, 1 mL was sub-sampled and further diluted in a 1 : 10 solution containing 20% nitric acid and 5 ppm yttrium (approx. 500x final dilution). Both dilutions were run on a radial inductively coupled plasma atomic emission spectrometer (Vista, Varian, Inc., Palo Alto, CA) for determination of individual mineral

concentrations. The Co, Cu, Mo, and Se were reported from the 50x dilution, whereas Ca, K, Mg, Mn, P, S, and Zn were reported from the 500x dilution. Mineral samples were weighed, then digested in individual sealed vessels with nitric acid at a 1 mL acid : 50 mg mineral ratio, and then placed in an oven over-night at 95°C. The next day, 5 ppm of yttrium was added to the digested samples as an internal standard, and then the samples were diluted to a final volume of 50 mL (approx. 100x dilution) with Type 1 H_2O. From each digested and diluted sample, 1 mL was sub-sampled and further diluted in a 1 : 10 solution containing 20% nitric acid and 5 ppm yttrium (approx. 1000x final dilution). Only the 1000x dilution was run on the radial inductively coupled plasma atomic emission spectrometer for determination of individual mineral concentrations. All elements were reported from the 1000x dilution. From the water samples, 9.4 mL was aliquotted and combined with 1 ppm yttrium and 500 ppm cesium for a final volume of 10 mL. The sample was then run on an axial inductively coupled plasma atomic emission spectrometer (Vista, Varian, Inc., Palo Alto, CA) for determination of individual mineral concentrations.

Serum Urea Nitrogen. Frozen serum samples were allowed to thaw for 24 h at 4°C, then analyzed for determination of urea nitrogen concentration using the QuantiChrom™ Urea Assay Kit (DIUR-500, BioAssay Systems, Hayward, CA). Optical density of urea was determined using a 96-well multi-detection microplate reader (Synergy™ HT, BioTek® Instruments, Inc., Winooski, VT), and then urea-N concentration (m*M*) was determined using a standard curve calculation.

Trace Mineral Status. Liver biopsy samples were shipped over-night in coolers with ice packs to the aforementioned commercial lab (Michigan State University,

Diagnostic Center for Population and Animal Health, Lansing, MI). Hepatic trace mineral concentrations were determined as previously described (Wahlen et al. 2007; Stowe et al. 1985 and 1986). Upon arrival, each liver biopsy sample was weighed, dried over-night at 75°C, and then reweighed for determination of dried biopsy weight. Then, the biopsy samples were digested in sealed vessels containing a ratio of 1 mL nitric acid : 125 mg liver tissue and placed in an oven over-night at 95°C. The digested samples were then diluted at a 1 mL Type 1 H_20 : 10 mg liver tissue ratio (approx. 100x dilution). Two-hundred μL of each digested and diluted sample was then further diluted in a 1: 20 solution containing 0.5 % EDTA and Triton X-100, 1.0 % ammonia hydroxide, 2.0 % propanol, and 20 ppb of scandium, rhodium, indium and bismuth (used as internal standards). All samples were analyzed using inductively coupled plasma mass spectrometry (Agilent 7500ce, Agilent Technologies, Santa Clara, CA) for determination of individual mineral concentrations.

Statistical Analysis

The trial was conducted as a randomized complete block (age of cow) design with repeated measures. Pastures of cows (or replicates) were treated as the experimental unit and individual cow as the sampling unit. The BW, BCS, SUN, and hepatic trace mineral concentration data from the cows were analyzed as repeated measures using mixed model procedures (SAS Inst. Inc., Cary, NC). The model included the fixed effect of treatment (CON, DDG), data collection day (d -8, 28, 54, and 77 depending on dependent variable, as a repeated measure), the interaction of treatment by collection day, and the two covariates of calving and treatment exposure. The random portion of the model included

the effects of replicate nested within treatment and cow age (young versus mature cows; designated as the blocking factor), cow nested within replicate, and the residual variance. When treatment by data collection day interaction was nonsignificant ($P > 0.05$), main effects were tested and presented. Covariates for cow data analysis were: 1) days of exposure to treatment and 2) days between cow removal from treatment and parturition. For analysis of calf birth weight, SUN, and hepatic mineral concentrations of the calves, the model included covariates, the random effect of block, and the fixed effect of treatment. Covariates for calf data analysis were: 1) days between cow removal from treatment and parturition and 2) days between calf birth and liver biopsy. For all models, residual and box plots were used to examine normality, goodness of fit statistics were used to choose the repeated measures structure, random sources of variation with zero or near zero variance were removed and non-significant interactions were deleted from the model. Tukey's comparison procedure was used to test differences between covariate corrected least squares means if significant (or tendencies of) main effects or interactions were found. Significance was declared at $P \leq 0.05$, and a tendency at $0.05 < P \leq 0.10$. For higher levels of significance, results are reported as $P \leq 0.01$.

RESULTS and DISCUSSION

Background. Fuel ethanol has become a booming industry in those countries where agricultural and economic conditions are favorable; first in Brazil in 1975, followed by the USA in 1978, and then more recently, Canada (Wheals et al. 1999). Feedstocks such as corn, sorghum, barley, wheat, sugarcane, sugar beets, and other lignocellulosic crops (or wood fibers) can be used on a large-scale to produce fuel ethanol from the fermentation of starch by yeast (*Saccharomyces cerevisiae*). The by-products

from ethanol production consist of a thin liquid referred to as "stillage" or "liquor" and a dense particulate mash referred to as "distiller's grains." These by-products can either be separated by centrifugation and sold as livestock feeds, known separately as condensed distiller's solubles or as "wet" or "dry" (dehydrated) distiller's grains, or recombined and sold as either "wet" or "dry" (dehydrated) distiller's grains with solubles (Loy 2007).

To allow for the yeast to be reused for multiple batches and as a measure of sterilization and pH control, dilute sulfuric acid ($\mathbf{H_2SO_4}$) is used during ethanol production. However, this rinsing with H_2SO_4 may increase the concentration of S in distiller's by-products. When consumed by livestock, high levels of dietary S can decrease the availability of other minerals and elicit mineral deficiencies, most notably Cu (Goodrich and Tillman 1966; Suttle 1974).

In addition to the increased concentration of S, other nutrients become more highly concentrated in distiller's grains compared to the grain from which it originally came. For example, starch (the fraction removed during ethanol production) comprises nearly two-thirds of the corn kernel, so when it is removed, the distiller's by-product is 3-fold more concentrated in protein, fat, fiber, and P than corn itself. Klopfenstein et al. (2008) previously outlined these nutrient changes: protein typically 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. These increases in protein and energy make distiller's grains an attractive feedstuff for livestock, especially when the product is priced competitively among alternative protein and energy commodities, but may contribute to inconsistencies in nutrient concentration from batch to batch and may lead to an aberrant provision of nutrients.

In the current study, 6 separate loads of DDGS were received during the 77 d that the cattle were exposed to treatment. The CP ranged from ~37 to 38 %, fat from ~14 to 18%, NDF from 40 to 47%, P from 0.93 to 0.98%, and S from 0.71 to 0.77% (DM). It is also worth noting that the NDFICP and the ADFICP (Table 2) were higher in our DDGS samples than what is reported in the NRC (1996); ranging from ~45 to 55 and from ~32 to 46 % of CP, respectively. However, the NDFICP values reported are similar to the escape protein values previously reported for DDGS by Ham et al. (1994). These high levels of NDFICP and ADFICP indicate that ~32 to 46 % of CP in the DDGS used in the current study may not have been biologically available (Van Soest 1994), which was not expected during diet design and could consequently reflect in protein deficiencies in the cows and their calves.

Seven of the 216 calves were not born alive, and 1 cow from the liver biopsy subset from the CON treatment was removed at the end of the study due to the loss of her calf.

Intake. It has been previously reported that the intake of grazed forages will range from 0.91 to 4.3% of BW in cattle grazing native grass range (Caton and Dhuyvetter 1997). However, because the young cows were offered 3.02 kg cow^{-1} d^{-1} (DM) of the triticale hay throughout the study and the mature cows were not, the total calculated DMI for young cows was numerically higher than mature cows. This additional delivery of roughage to young cows was intended to provide additional energy, as it was assumed that they had a higher NE_m requirement (NRC 1996) than the mature cows in this study. In contrast, the mature cows were only sporadically offered sorghum hay, as a replacement for inaccessibility to pasture during heavy snow fall; therefore it

was not included in the calculated nutrient intake of cows shown on Table 3. On those days (12 days over the course of the study), 5.82 kg cow^{-1} d^{-1} (DM) of the sorghum hay was delivered. It seems likely that the use of a young versus mature cow blocking factor accounted for this variation in delivery of feedstuffs and the subsequent difference in calculated DMI between the two age groups.

Actual intake of mineral for both treatments exceeded target intake. Average mineral disappearance was 127 and 162 g cow^{-1} d^{-1} (DM) for the CON and DDG cows, respectively, vs. the 110 g cow^{-1} d^{-1} (DM) intended target intake for both groups. Consequently, DDG cows consumed ~28% more mineral than the CON cows and ~47% more mineral than intended by study protocol. The amount of daily mineral disappearance indicated that all cows consumed enough mineral to meet their NRC-recommended requirements of 10 mg of Cu, 30 mg of Zn, 50 mg of Fe, 0.10 mg of Co, and 40 mg of Mn/kg of DM (NRC, 1996). Factors that may account for increased loose mineral intake by both groups include ad libitum availability, higher palatability than expected, high intake by dominant cows, boredom, or hunger; all of which could be attributed to the conditions of the dormant winter pastures they were maintained on. Additionally, both minerals contained equal amounts of salt (16.5 %, as-fed; data not shown) and DDGS was used as the carrier in both. While not measured in the current study, it seems reasonable to relate the higher-than-expected mineral consumption of DDG cows with the elucidation that feeding DDGS may have attributed to increased DMI. Recently, Loy et al. (2007) reported that heifers offered DDGS, on a daily-basis, had increased DMI (2.12% of BW), compared to controls that received no supplementation (1.88 % of BW). Furthermore, the high mineral intake by the DDG

cows could also be attributed to a lower P concentration in the DDG mineral mix. The DDG mineral was purposely formulated for a lower concentration of P, as we had calculated that the intake from DDGS would provide adequate P. However, a lower concentration of P will increase palatability and intake (Peterson et al. 2005). Lastly, while it was intended for there to be no difference in trace mineral concentration between the 2 mineral supplements, there appeared to be differences upon analysis, particularly in Cu, Zn, and Fe (Table 2). Regardless of the excess mineral intake, there appeared to be no deleterious effects on cattle health or performance.

Cow Performance. Mean BW of all cows increased throughout the 85 - d collection period (Figure 1a), which is most likely attributed to third-trimester fetal calf growth during this time. However, DDG-supplemented cows had higher BW than CON cows as calving approached (Figure 1a). Additionally, DDG cows had higher (data not shown; $P = 0.01$) ADG compared to CON cows (0.48 versus 0.17 kg, ± 0.06 kg, respectively) over the course of the study. Gestating cows should be expected to gain between 0.28 and 0.77 kg/d during this period (NRC 1996). Also, gain as a percentage of BW was higher in DDG cows (data not shown; $P = 0.01$) than CON cows (5.34 vs 2.13 %, respectively). These data indicate that the DDG diet was likely more sufficient at providing minimum requirements of NE_m (Mcal/d; NRC, 1996) than the CON diet as calving approached.

In contrast to the increase in BW, there was a decrease ($P < 0.001$) in mean BCS for all cows as the time of calving approached (Figure 1b). Mean BCS was higher (treatment * day, $P < 0.01$) in DDG-supplemented cows on d 28 and 54, therefore indicating that body condition was better maintained through the winter when DDGS

were supplemented. The clearest explanation of why DDG cows had less BCS loss than the CON cows on d 54 and 77 was likely due to higher intake of DM, protein, and energy as calculated on Table 3. While actual intake was not measured, this difference in nutrient provision would likely provide a greater plane of nutrition and help to maintain a higher BCS through the harshest winter months. Regardless, there was no difference in BCS between cows at the end of study (d 77), indicating that pasture quality and availability was likely deficient and forage intake was consequently low by d 77. Furthermore, it has been suggested that ruminants managed on low quality, dormant forages are likely to have increased energy expenditure associated with the work of grazing as the availability of forage decreases. As grazing time and energy needs increase and forage availability decreases, energy expenditure for maintenance increases (Caton and Dhuyvetter 1997). This could be especially true when compounded with the elevated energy needs during the third trimester of gestation (NRC 1996). It is likely that the amount of supplement being offered to either group of cows was not sufficient in preventing loss in body condition as the time of calving approached. Therefore, increasing the amount of energy available in the diet toward the end of the study, either by increasing feeding rates of either the supplements or hay or both, may have served well to better maintain body condition.

If the strategy to increase feeding rates of supplements such as DDGS to prevent BCS loss during the last 3rd of pregnancy is employed, doing so should be done conservatively. It has been previously indicated that DDGS should not be fed above ~3 kg cow^{-1} d^{-1} (DM) as the high-fat content may cause a reduction in forage digestion (Klopfenstein et al. 2008). Additionally, increasing the level of supplement can decrease

the amount of time spent grazing (Caton and Dhuyvetter 1997), so increasing the level of supplementation should be closely managed. In the current study, the DDGS were limit-fed for a target intake of 1.54 kg cow^{-1} d^{-1} (DM). Therefore, it seems likely that the feeding rate of either supplement could have been increased as cows approached calving. This strategy would help cows maintain BCS, particularly as the availability of winter range simultaneously became sparser through the winter months of the study.

Serum Urea Nitrogen Status. Serum urea nitrogen (**SUN**) is a convenient measure of protein status in ruminants (Huntington and Archibeque 2000). The pattern for SUN concentration was different for the two treatment groups over the course of the study (treatment * day interaction, $P < 0.01$; Figure 1c). On d - 8, the mean SUN concentration of CON-supplemented cows was significantly lower than the DDG-supplemented cows, and was otherwise not different on subsequent data collection days. There is no clear explanation for the disparity on d - 8, as all of the cows had been pastured together and fed the same diet before the start of the study. In agreement with the similarity in mean SUN concentration between treatment groups on d 54 and 77, Nichols et al. (1998) reported no difference for plasma urea nitrogen when lactating dairy cows were fed a dietary treatment scheme similar to that in the current study (corn distiller's grains compared to a control diet with soybean meal). The similarity in mean SUN concentration between treatment groups in our study data indicates that the variability in NDFICP and ADFICP between the CON and DDG supplements was utilized similarly by pregnant range cows. However, Ferguson et al. (1988, 1993) reported that SUN concentrations exceeding 6.66 m*M* (20 mg/dL) were associated with reduced conception rates in lactating dairy cows. Comparatively, the mean SUN

concentrations in all cows from our study ranged between 6.40 to 9.75 m*M*, which could indicate that the nitrogen status of all cows, regardless of treatment, may have been in excess.

The basic concepts of protein metabolism have been previously reviewed (Klopfenstein 1996; Huntington and Archibeque 1999) and will be relied upon for this discussion. Briefly, dietary CP offered to ruminants can be divided into nitrogenous components that can be used to support the synthesis of microbial cell protein, are absorbed by the small intestine and used for protein metabolism in the host, or are completely non-degradable and indigestible. These protein fractions can be generally classified into two groups: soluble nonprotein nitrogen, which can also be referred to as ruminally degradable intake protein (**DIP**), or the insoluble, undegradable intake protein (**UIP**). In the current study, NDFICP and ADFICP were used as estimates for the % of CP that was UIP, whereas DIP was estimated to be the balance (%) of CP that remained when NDFICP (and ADFICP, therewithin) were subtracted from total CP.

As seen in our nutrient composition analysis (Table 2) and in previous studies (Krishnamoorthy et al. 1982; Kleinschmit et al. 2006; MacDonald et al. 2007), the CP in DDGS is approximately 50% rumen UIP. This value was estimated from the balance of CP that would remain when the ~49.29% value is subtracted from total CP to account for NDFICP (and ADFICP). Based off of that estimation, it could be assumed that the DDGS fed in the current study contained approximately equal proportions of DIP and UIP. This uniform combination of DIP and UIP in DDGS makes the co-product an appealing protein source, able to advocate the N needs of both microbes and the host animal (Chalupa 1975; Waller et al. 1980; Klopfenstein 1996; Huntington and

Archibeque 1999). In comparison, the ratio of DIP to UIP in the range cubes could be estimated at approximately 60 : 40. In a review, Chalupa (1975) suggested that production in ruminants is often improved when feeds higher in 'escape proteins' (or UIP) are digested post-ruminally. More recently, Loy (2007) indicated that increasing the level of UIP is most beneficial to ruminants when energy or protein intake is insufficient. Perhaps the higher level of UIP in the DDGS in the current study could further explain why DDG-supplemented cows gained more weight than the CON cows. However, our nutrient analysis also indicates that 39.71 % of the potential UIP in DDGS is bound in ADFICP, which has been regarded as essentially indigestible protein (Chaudry and Webster 1993). Additionally, Van Soest (1989) demonstrated a negative association between amount of ADFICP and nitrogen digestibility. Presumably, the ADFICP in the DDGS used in the current study contained protein products that may have been absorbed in the small intestine, but not utilized by tissues and was otherwise lost in urine (Van Soest 1994). This may explain why the SUN in both treatment groups was > 6.66 m*M* (Ferguson et al. 1988, 1993); the nitrogen provided in the diet of both groups may have been in excess.

Trace Mineral Status. A treatment * day interaction was detected in hepatic Fe ($P = 0.02$), Cu ($P < 0.01$), Co ($P = 0.05$), and Mn ($P < 0.01$) concentrations (Figures 2a, b, c, and d, respectively). Whereas Cu, Co, and Mn concentrations were elevated in the liver of DDG cows at the end of the study (d 77), Fe was elevated in CON cows on the same day. The higher hepatic Fe concentration in the CON cows is likely due to the higher level of Fe intake in the CON vs. DDG cows, as shown in Table 3. Regardless, the hepatic Fe status of both treatment groups were adequate, and may have even exceeded

adequacy in the CON cows on d 77 (Michigan State University, Diagnostic Center for Population and Animal Health, Lansing, MI). No treatment * day interaction was detected in hepatic Zn, Se, or Mo concentrations (Figures 3a, b, and c, respectively). Cows supplemented with DDGS tended ($P = 0.08$) to have increased liver Zn concentration on d 77 compared to d -8 (Figure 3a), whereas no treatment effect was found in hepatic Se or Mo status (Figures 3b and c, respectively).

The elevated hepatic trace mineral status seen in DDG cows was likely due to the higher-than-expected quantity of loose mineral consumption by DDG cows. As aforementioned, the mineral intake for both treatments exceeded target intake, however DDG cows far exceeded expected intake (consuming ~28% more than CON cows and ~47% more than intended). Table 3 shows that DDG cows consumed greater amounts of Cu, Zn, Co, and Mn, which would explain the significantly and numerically elevated Cu, Zn, Co, and Mn status reflected in DDG cows on d 77. The hepatic Cu, Zn, Co, and Mn concentrations were 110, 16, 73, and 37 %, respectively, higher in DDG cows than CON cows.

One of the main objectives on this study was to demonstrate the decrease in Cu status in cows supplemented with DDGS due to elevated S intake (~14 vs. ~20 g cow^{-1} d^{-1} in CON and DDG cows, respectively). In the current study, we calculated that the daily S intake cow^{-1} d^{-1} averaged ~0.09 and 0.06 % of DMI for young and mature CON cows, respectively, and 0.14 and 0.13 % of DMI for young and mature DDG cows, respectively (calculated from estimated values presented in Table 3). However, when S intake was calculated for day of supplement delivery, rather than averaged across day, these values are higher. On day of feeding, S intake was 0.11 and 0.16 % of DMI for young and

mature CON cows and 0.21 and 0.27 % of DMI for young and mature DDG cows, respectively. It is also worth noting that the forage samples collected were quite low (0.07 and 0.08 %, for young and mature cow pasture, respectively) in S relative to requirements of cattle fed forage based diets. The S requirement of mature cows in gestation or lactation is 0.15% of dietary DM (NRC, 1996). Therefore, it is plausible that the CON cows did not receive adequate dietary S, and it could therefore be elucidated that the S supplied from the DDGS may have improved ruminal fermentation and forage intake in the DDG cows in the present study.

While sulfur-based compounds are essential in the ruminant diet, for the purpose of microbial *de novo* sulfur-amino acid synthesis, high levels of dietary S can be problematic. One of the most sensitive mineral interactions involves the antagonism of excess S on Cu (Goodrich and Tillman 1966; Suttle 1974; Kandylis 1983). While the Cu status of beef cattle varies with age and level of productivity, the normal range for hepatic Cu concentration in mature cows is 50-400 mg / kg (Mills 1987). In previous work, addition of S to the ruminant diet (above 0.3 to 0.4%) has decreased plasma (Suttle 1974) and hepatic Cu concentrations (Goodrich and Tillman 1966), which can lead to decreased feed intake and performance (Kandylis 1983), cause over-production of hydrogen sulfide (H_2S) gas in the rumen, depress rumen motility and cause thiamine deficiency (Edwin et al. 1968), and can ultimately cause severe distress of the nervous system, known as polioencephalomalacia (Gould 1998).

While the intake of S in the current study did not reach those levels described above, we did expect to see an antagonism between S intake and Cu status. However, contradictory to our hypothesis, the Cu status in DDG cows improved over the course of

the study, while hepatic Cu status in the CON cows lessened (Figure 2b). Perhaps this contradiction could be explained either by the higher than expected Cu consumption by DDG cows, or because CON cows consumed higher Fe and Mo than the DDG cows. Aside from S, other antagonists such as Mo and Fe have been shown to induce hypocuprosis in ruminants (Bailey et al. 2001). Copper status has been shown to decrease with elevated dietary Fe intake (Gengelbach et al. 1994) and can be rendered unavailable by the highly reactive thiomolybdate complexes formed in the sulphide-rich environment of the rumen (Suttle 1991). The range cubes and loose mineral mix offered to CON cows were inherently higher in Fe and Mo, so there is a possibility that that the combination of both Fe and Mo antagonized Cu status in CON cows.

Iron status also influences Zn, Co, and Mn absorption. While there were no differences in hepatic Zn status found, there was a numerical increase in Zn status in the DDG cows (129.0 vs. 149.5 mg / kg of DM in CON and DDG cows, respectively) on d 77. It has been shown that Zn absorption is enhanced when dietary Fe is decreased (Bremner and Mills 1981). In the current study, CON cows had significantly higher Fe status, presumably due to higher calculated Fe intake. This difference in calculated Fe intake, likely explains the numerical difference in Zn status between the two treatment groups as time of parturition approached. Additionally, a mutual antagonism exists between Fe and both Co and Mn absorption in the small intestine (Thomson and Valberg 1972; Ho et al. 1984). It is likely that the higher Fe intake, combined with lower Co and Mn intake of CON cows is the explanation for the significant difference ($P < 0.05$) in liver Co and Mn concentrations between treatment groups on d 77.

All cows were fed the same pasture and a loose commercial mineral mix before the start of the study; this diet may have impacted their initial mineral status. For example, none of the cows appeared to be deficient in Fe, Cu, Se, or Mo, but were close to deficiency levels in Zn, Co, and Mn (Michigan State University, Diagnostic Center for Population and Animal Health, Lansing, MI). Nevertheless, supplementing with DDGS did not have an antagonist effect on the Cu status in cows, but rather may have elevated loose mineral intake and contributed to the enhanced hepatic trace mineral status.

Cost of Production. There was a cost-benefit in feeding the DDG diet over the CON diet to the late-gestation cows. At the time of the study, the price / t (DM) of feedstuffs (excluding delivery charges) were as follows: hay, $97; sunflower-based range cubes, $298; CON mineral, $665; DDGS, $201; and DDG mineral, $450. Therefore, not including the cost of pasture, the feeding cost cow^{-1} d^{-1} (based on actual disappearance of hay, supplements, and mineral offered) was $0.90 for young CON cows, $0.58 for mature CON cows, $0.72 for young DDG cows, and $0.40 for mature DDG cows. This difference between treatments equates to a net savings of $13.86 / cow over the duration of the 77 - d feeding period when the DDGS were fed.

It is also worth noting that the range cube and DDGS supplements were offered on alternating days (3x each wk), rather than daily, due to extensive grazing conditions. While Loy et al. (2007) indicated that supplementing with either dry-rolled corn or DDGS on alternating days (every other day) rather than daily resulted in depressed forage intake (and altered intake pattern), it has also been reported that high-protein supplements can be fed less frequently than daily without significant negative effects on livestock performance (Wallace 1988; Collins and Pritchard 1992; Bohnert et al. 2002; Schauer et

al. 2005). Delivering feed to range cows on alternating days could be an advantage to cow-calf producers, particularly when cost of labor, fuel, and use of equipment is factored into the cost of delivering feed. Therefore, decreasing the number of days that feed would have to be delivered, could reduce feed costs during winter months, and if well-managed have no negative impact on cow performance.

Calf Variables. Calf birth weight tended ($P = 0.10$) to be greater in calves born to cows supplemented with DDGS (Table 4). This trend for increased birth weight in the calves of DDG cows supports findings from previous work (Beaty et al. 1994) that supplemental CP intake in gestating cows advocates the amino acid and gluconeogenic precursors needed for fetal growth and metabolism. While both CON and DDG cows received supplemental protein, it is likely that the increased intake of both CP and energy by DDG cows compared to CON cows (as calculated in Table 3) may have contributed to increased birth weight in calves born of DDG-supplemented cows. While exposure to dietary treatment ended for all cows by d 77, weaning weights for calves were recorded on Sept. 3, 2008. There was no difference ($P = 0.41$) in calf weaning weight due to dietary treatment (Table 4). This data indicates that the difference in birth weight due to maternal dietary supplementation became ambiguous by the time the calves were weaned. Further research is needed to determine what effects, if any, on calf performance may occur if DDGS are supplemented through time of weaning.

No differences ($P \geq 0.39$) were observed in SUN concentration or of hepatic mineral concentrations in calves (Table 4). It is worth noting, however, that the hepatic Zn and Cu concentrations in the calves were both below what is considered adequate for neonatal calves and were near deficiency levels (Engle et al. 1997; Michigan State

University, Diagnostic Center for Population and Animal Health, Lansing, MI). This could be due to the extremely high liver Mo concentration in the calves. Typical neonatal calf liver Mo concentrations range between 0.4 - 0.9 mg / kg (Michigan State University, Diagnostic Center for Population and Animal Health, Lansing, MI), whereas the calves in our study were 3 to 6-fold times above that range (2.07 and 2.69 mg / kg in CON and DDG calves, respectively). Previously, Muehlenbein et al. (2001) collected liver tissue samples from cows and their calves at 10 and 30 d post-calving following trace mineral supplementation and reported hepatic Zn values below ours (range of 92 to 194 mg / kg, DM in 10 d old calf liver samples), but the Cu values were similar (106 to 249 mg / kg, DM in 10 d old calf liver samples).

While Mo was not added to any of the dietary treatments in the current study, both the range cubes and the DDGS inherently contained trace amounts (2.7 and 1.1 mg / kg, respectively). Genglebach et al. (1994) demonstrated that adding Mo to the diet of cows and their calves decreased Cu status. In that study, calves were measured for plasma Cu concentrations from d 7 to 280, post-calving. They found no difference due to treatment in 7 d old calves, however calves fed the Mo treatment had lower plasma Cu concentrations than control calves as they aged. Additionally, a relatively low concentration of Mo in the diet (the addition of 5 mg / kg to diets containing 0.1 mg / kg of Mo) has been shown to cause Cu depletion in beef cows and their calves (Bremner et al. 1987; Phillippo et al. 1987a,b). Therefore, it seems likely that while the calves in the current study were not fed the treatment diets as the calves had been in those trials mentioned above, there is a possibility that the combination of trace amounts of Mo along

with the S intake from both the CON and DDG cows led to a below adequate Cu status in the calves.

Summary. In summary, this experiment demonstrated that limit-fed, short-term supplementation of DDGS through the last trimester of gestation improved performance, and had no deleterious effects on nitrogen or mineral status on either the cows or their calves. Furthermore, given the reduced feeding costs, it would be economically justifiable to offer a higher feeding rate of the DDGS given that protein, fat, and S intake is closely managed. Feeding more DDGS could potentially afford the cow-calf producer better maintenance of body condition in the cow herd and coincidentally support more ADG during the last third of pregnancy, which in the current scenario was exacerbated by harsh winter weather. Additionally, fuel and labor expenses associated with feed delivery could potentially be reduced when supplements are delivered on alternating days (3 times each week) instead of every day without any negative effects on cow performance.

The difficulty in performing a range cow study is not only the variability in weather patterns and the subsequent effect on pasture forage consumption, but also the interval in which the cows calved. The expected calving date of each cow had been previously estimated using rectal palpation before the start of the trial. Approximately 63% of the cows were expected to calve between d 66 and 82 of the study (average, d 74), and were consequently grouped as "early" calving cows and removed from treatment on d 54, whereas the ~ 37% of cows that remained were expected to calve between d 83 and d 120 (average, d 92), were removed from treatment on d 77, and grouped as "late" calving cows. This difference in calving interval makes it difficult to elucidate the effect that treatment had on the aforementioned parameters in the calves because some of the

later calving cows were removed from treatment up to 43 days before calving, whereas some of the earlier calving cows were removed from treatment 1 d before calving (there were cows that calved earlier than expected, and before the d 54 removal from treatment). While we used covariates to account for some of this variability, it is likely that we were not able to find differences in calf SUN or hepatic trace mineral status due to this variation in treatment exposure of their dams. It is also likely that the age of the calves (< 1 wk of age), at the time of sample collection, was not the appropriate age for best assessing potential differences in hepatic mineral status or of SUN concentrations. Also, allowing ad libitum mineral intake, rather than offering a more fixed or more controlled amount, was likely reflected in the results obtained.

When distiller's grains are competitively priced (on a DM basis) among alternative protein and energy supplements, cow-calf producers should consider not only the cost benefit but the additional performance that could be achieved by feeding DDGS. Future research is needed to establish what the effects on performance, SUN, and hepatic mineral status would be on cows and their calves when fed for a complete gestation-lactation cycle, and as the calves go through the weaning, growing, and finishing stages.

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TABLES AND FIGURES

Table 1. Nutrient composition (DM-basis) of basal feedstuffs offered to all cows in the study[z,y]

Nutrient	Basal Feedstuffs				
	H_2O	Young Cow Pasture[x]	Mature Cow Pasture[x]	Young Cow Triticale Hay[x]	Mature Cow Sorghum Hay[x]
DM, %	ND	77.3 ± 14.4	85.4 ± 9.1	87.5 ± 0.9	88.3 ± 0.7
CP, %	ND	3.8 ± 0.7	5.2 ± 0.9	10.7 ± 0.6	9.9 ± 1.2
NDFICP, % of CP[w]	ND	26.2 ± 18.0	17.0 ± 17.7	25.7 ± 6.9	57.2 ± 10.0
ADFICP, % of CP[w]	ND	12.5 ± 9.3	14.6 ± 9.3	21.3 ± 10.2	16.3 ± 4.8
NDF, %	ND	76.4 ± 1.3	78.1 ± 1.8	64.6 ± 5.3	70.1 ± 2.6
ADF, %	ND	42.1 ± 0.5	42.5 ± 1.4	32.4 ± 0.6	43.4 ± 1.2
NFE, % [v]	ND	7.2 ± 1.2	4.9 ± 3.0	10.4 ± 5.3	5.0 ± 2.8
TDN, % [u]	ND	50.5	50.5	64.0	56.1
DE, Mcal/kg[u]	ND	2.23	2.23	2.82	2.47
ME, Mcal/kg[u]	ND	1.83	1.83	2.31	2.03
NE_L, Mcal/kg[u]	ND	1.12	1.12	1.45	1.25
NE_M, Mcal/kg[u]	ND	0.99	0.99	1.44	1.18
NE_G, Mcal/kg[u]	ND	0.44	0.44	0.86	0.62
Ash, %	ND	5.6 ± 1.9	5.3 ± 1.4	6.9 ± 0.8	9.3 ± 0.4
Ca, %	ND	0.25 ± 0.02	0.29 ± 0.04	0.18 ± 0.01	0.41 ± 0.05
P, %	ND	0.08 ± 0.01	0.08 ± 0.01	0.23 ± 0.05	0.19 ± 0.02
Mg, %	0.003 ± 0.001	0.069 ± 0.005	0.066 ± 0.001	0.114 ± 0.005	0.284 ± 0.035
K, %	ND	0.24 ± 0.05	0.26 ± 0.04	1.73 ± 0.22	2.54 ± 0.27
Na, %	0.012 ± 0.008	0.006 ± 0.002	0.005 ± 0.002	0.001 ± 0.001	0.003 ± 0.001
S, %	0.008 ± 0.001	0.072 ± 0.005	0.082 ± 0.008	0.132 ± 0.009	0.126 ± 0.012
Cu, mg / kg	ND	10 ± 9	12 ± 16	5 ± 1	7 ± 1
Zn, mg / kg	ND	23 ± 8	24 ± 10	20 ± 1	32 ± 4
Fe, mg / kg	ND	290 ± 89	253 ± 56	152 ± 54	160 ± 12
Co, mg / kg	ND	ND	ND	ND	ND
Mo, mg / kg	ND	ND	ND	ND	ND
Mn, mg / kg	ND	34 ± 2	37 ± 10	37 ± 2	35 ± 5

[z] Nutrient values presented as means plus or minus the standard deviation.

[y] ND = not detected.

[x] Cows were blocked and separated into different pastures by age, where "young" cows = 3 yrs-of-age and "mature" cows were > 3 yrs-of-age. The young cows were rotated through a set of pastures that were separate from those that the mature cows were rotated through. The young cows were also offered 3.03 kg cow^{-1} d^{-1} of triticale hay throughout the study, whereas the mature cows were only fed approx. 5.82 kg cow^{-1} d^{-1} of sorghum hay on days of extreme winter conditions (DM basis).

[w] NDFICP = NDF insoluble crude protein (fraction of the crude protein that is neither assumed to be instantly degradable nor completely undegradable; associated with NDF fraction of the diet). ADFICP = ADF insoluble crude protein (fraction of crude protein that is estimated to be completely undegradable; associated with the ADF fraction of the diet). Both are reported as a percentage of CP.

[v] NFE = nitrogen free extract; determined by difference.

[u] Energy values (TDN, DE, ME, and $NE_{L,M,G}$) were calculated, based off of NRC (1996) values. No standard deviations available to report.

Table 2. Nutrient composition (DM-basis) of supplements offered to control cows (**CON**) or to cows fed dried distiller's grains with solubles (**DDG**)[z,y]

	Treatment			
	CON		DDG	
Nutrient	Range Cubes	Mineral[x]	DDG	Mineral[x]
DM, %	87.9 ± 2.3	97.6	91.3 ± 3.9	97.0
CP, %	33.7 ± 0.7	4.2	37.1 ± 0.5	8.0
NDFICP, % of CP[w]	40.4 ± 1.7	ND	49.3 ± 4.7	31.5
ADFICP, % of CP[w]	18.5 ± 6.0	ND	39.7 ± 5.7	ND
Crude Fat, %	5.7 ± 0.2	2.8	15.5 ± 1.7	2.2
NDF, %	50.0 ± 6.9	7.1	44.3 ± 2.6	12.2
ADF, %	17.2 ± 0.1	1.5	14.2 ± 1.2	3.4
NFE, %[v]	4.5 ± 6.2	19.7	ND	24.1
TDN, %[u]	65.00	-	88.00	-
DE, Mcal/kg[u]	2.87	-	3.88	-
ME, Mcal/kg[u]	2.35	-	3.18	-
NE_L, Mcal/kg[u]	1.47	-	2.04	-
NE_M, Mcal/kg[u]	1.47	-	2.18	-
NE_G, Mcal/kg[u]	0.88	-	1.50	-
Ash, %	5.6 ± 0.5	65.7	4.3 ± 1.0	52.8
Ca, %	0.40 ± 0.01	13.84	0.03 ± 0.01	18.99
P, %	0.79 ± 0.02	9.34	0.95 ± 0.02	0.25
Mg, %	0.42 ± 0.01	2.33	0.32 ± 0.01	2.51
K, %	1.62 ± 0.04	1.69	1.20 ± 0.04	2.36
Na, %	0.08 ± 0.01	5.96	0.24 ± 0.06	7.63
S, %	0.33 ± 0.01	0.83	0.75 ± 0.01	0.61
Cu, mg / kg	20 ± 1	417	6 ± 1	797
Zn, mg / kg	65 ± 3	1640	64 ± 3	3010
Fe, mg / kg	233 ± 89	9850	97 ± 9	6027
Co, mg / kg	ND	36	ND	41
Mo, mg / kg	2.74 ± 0.12	ND	1.10 ± 0.03	ND
Mn, mg / kg	46 ± 1	2604	18 ± 2	3050

[z] Nutrient values presented as means, plus or minus the standard deviation.

[y] ND = not detected.

[x] Only one random sample of each of the loose mineral supplements was obtained during the study, hence there are no standard deviations available to report.

[w] NDFICP = neutral detergent fiber insoluble crude protein (fraction of the crude protein that is neither assumed to be instantly degradable nor completely non-degradable; associated with NDF fraction of the diet). ADFICP = acid detergent fiber insoluble crude protein (fraction of crude protein that is estimated to be completely non-degradable; associated with the ADF fraction of the diet). Both are reported as a percentage of CP.

[v] NFE = nitrogen free extract; determined by difference.

[u] Energy values (TDN, DE, ME, and $NE_{L,M,G}$) were calculated, based off of NRC (1996) values. No standard deviations available to report.

Table 3. Calculated average nutrient intake of cows offered either a control supplement of range cubes (**CON**) or dried distiller's grains with solubles (**DDG**)[z,y,x,w]

	Treatment			
	Control		DDG	
Nutrient	Young	Mature	Young	Mature
Total DMI, kg	10.70	8.74	11.03	9.00
CP, kg	1.06	0.88	1.13	0.94
Crude Fat, kg	0.55	0.44	0.72	0.59
NDF, kg	7.31	6.31	7.68	6.43
ADF, kg	3.78	3.28	3.99	3.35
NFE, kg	0.84	0.44	0.83	0.40
Ash, kg	0.71	0.54	0.73	0.54
TDN, kg	5.96	4.57	6.58	5.01
DE, Mcal	26.29	20.13	29.02	22.10
ME, Mcal	21.56	16.51	23.79	18.12
NE_L, Mcal	13.34	10.15	14.79	11.22
NE_M, Mcal	12.52	9.22	14.10	10.45
NE_G, Mcal	6.55	4.43	7.68	5.41
Calcium, g	28.98	23.55	36.65	31.22
Phosphorus, g	30.61	23.67	21.23	14.28
Magnesium, g	12.55	9.23	12.06	8.74
Potassium, g	78.72	26.48	73.61	21.36
Sodium, g	8.85	8.82	15.90	15.87
Sulfur, g	9.90	5.97	15.87	11.94
Copper, mg	158.54	168.29	219.57	226.45
Zinc, mg	505.28	476.44	794.64	758.20
Iron, mg	3813.41	3399.53	3508.43	2985.39
Cobalt, mg	4.57	4.57	6.64	6.64
Molybdenum, mg	4.05	4.05	1.61	1.61
Manganese, mg	717.08	662.82	858.56	793.48

[z]All nutrients are reported as total DMI of each nutrient cow^{-1} d^{-1}. Values reported are calculated and based on prediction equations. Total DMI was calculated by using the following equation: total DMI, kg = intake of NE_M (Mcal cow^{-1} d^{-1}) / average NE_M (Mcal/kg) concentration of pasture, hay, supplement, and mineral. Intake of NE_M was calculated using a prediction equation for pregnant beef cows (NRC, 1996) based on dietary energy concentration: intake of NE_M = shrunk body wt (**SBW**)$^{0.75}$ *(((0.04997 * (average NE_M^2 of feedstuffs not including pasture)) + 0.04631). The SBW was calculated using this formula: SBW = BW - (BW * 0.04). Where disappearance of delivered hay, DDGS or CON supplement, and mineral in each pasture replicated was known, disappearance of pasture was not measured. Therefore, pasture DMI was arrived at by subtracting DMI of hay, DDGS or CON supplement, and mineral from the total calculated DMI value.

[y] Diets consisted of range cubes (CON supplement) fed for a target intake of approximately 1.5 kg cow^{-1} d^{-1} (DM) or DDGS (DDG supplement) fed for a target intake of 1.46 kg cow^{-1} d^{-1} (DM), plus a unique loose mineral (to complement each supplement) provided for a target intake of 110 g cow^{-1} d^{-1} (DM). However, actual mineral intake was higher than expected, at 127 and 162 g cow^{-1} d^{-1} (DM) for CON and DDG cows, respectively.

[x] Cows were blocked and separated into different pastures by age, where "young" cows = 3 yrs-of-age and "mature" cows were > 3 yrs-of-age. The young cows were rotated through a set of pastures that were separate from those that the mature cows were rotated through. The young cows were also offered ~3 kg cow^{-1} d^{-1} (DM) of triticale hay throughout the study in an effort to maintain body condition. Mature cows were fed 5.82 kg cow^{-1} d^{-1} (DM) of sorghum hay only on days of extreme conditions (when the standing range was snow and ice covered), and is therefore not included for estimated intake calculations.

[w] Diets were formulated to meet daily TDN and CP minimum requirements for the last trimester of pregnancy (NRC, 1996).

Table 4. Birth weight, weaning weight, serum urea nitrogen (SUN), and liver mineral concentrations of calves born from cows fed either a control supplement (CON) or dried distiller's grains with solubles (DDG)[z,y,x]

	Treatment			
Item	CON	DDG	SEM	*P*-value[w]
Birth wt, kg[v]	38.89	40.62	0.69	0.10
Weaning wt, kg[u]	218.13	221.76	4.15	0.41
SUN, m*M*	17.73	19.93	8.06	0.80
----------Minerals, mg / kg of DM----------				
Fe	449.90	407.28	122.31	0.73
Zn	227.82	262.68	97.56	0.75
Cu	180.29	183.48	67.84	0.96
Se	2.23	2.31	0.59	0.90
Co	0.32	0.33	0.19	0.96
Mo	2.07	2.69	1.32	0.65
Mn	4.97	5.66	0.78	0.39

[z] n = 3

[y] Response variables are presented as covariate corrected least squares means.

[x] Data collected on calves born (aged < 7 days old) to a subset of 30 cows randomly selected for liver biopsies. One of the 30 cows lost her calf during pregnancy; n = 29 calves.

[w] Indicates the significance of difference detected between means within a row.

[v] Birth weight was recorded for all calves born alive from all cows in the study, N = 209.

[u] Exposure to dietary treatments ended for all cows by d 77, however weaning weight was recorded for all calves on d 126, N = 200.

Figure Legends

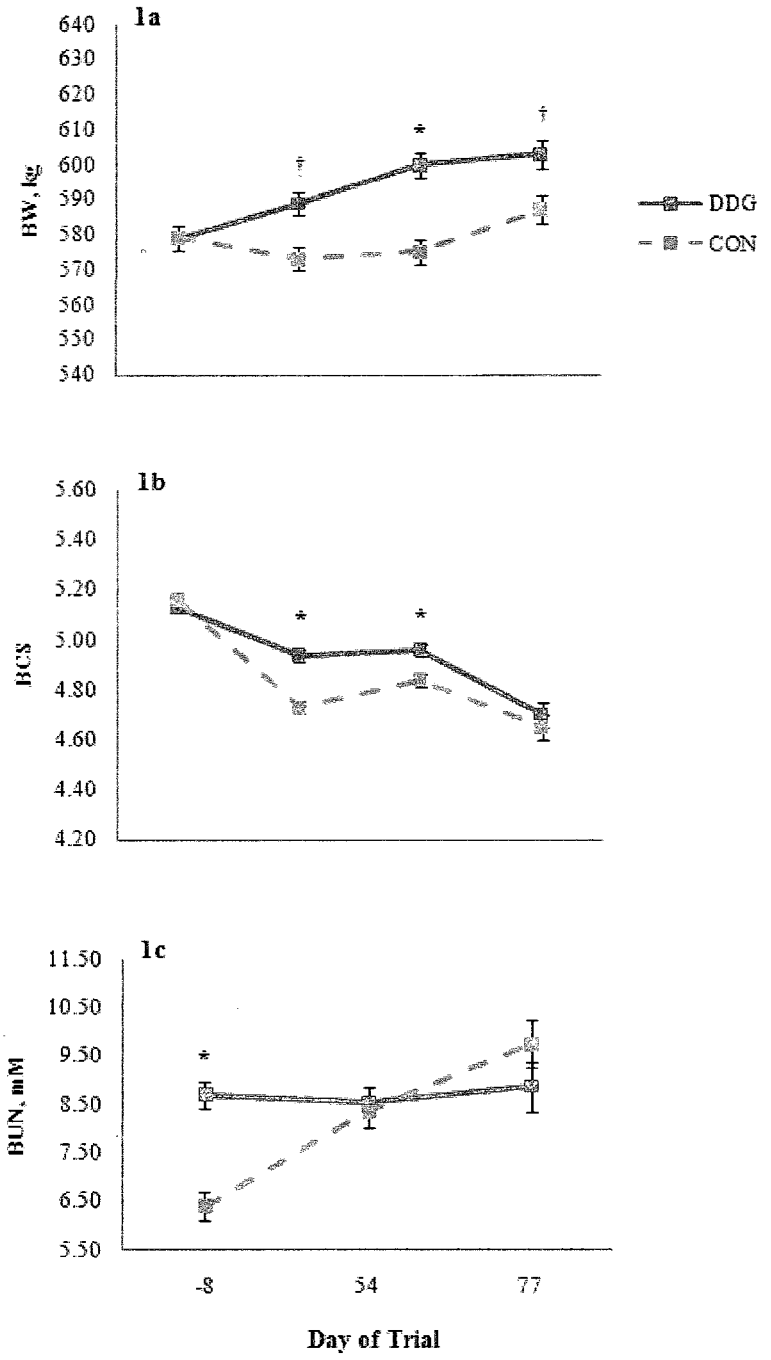
Figure 1 a - c. The BW (**1a;** treatment * day, $P < 0.01$), BCS (**1b;** treatment * day, $P < 0.01$), and serum urea nitrogen (**SUN**; **1c;** treatment * day, $P < 0.01$) concentration of cows fed either a control supplement (**CON**) or dried distiller's grains with solubles (**DDG**), n = 3. The BW and BCS data was collected on d - 8, 28 and 54 of the study with N = 216 and on d 77 with N = 79 total observations, whereas SUN data was collected on d - 8 and 54 of the study with N = 216, and on d 77 with N = 79 total observations. The data presented are covariate corrected least squares means. The * denotes differences between treatment means detected at $P \leq 0.05$, whereas † denotes differences between means detected at $0.05 < P \leq 0.10$. Standard error bars are attached to means, but are not apparent if smaller than the magnitude of the symbol.

Figure 2 a - d. Hepatic mineral concentrations of cows fed either a control supplement (**CON**) or dried distiller's grains with solubles (**DDG**), n = 3. The data presented are covariate corrected least squares means of hepatic Fe (**2a**), Cu (**2b**), Co (**2c**), and Mn (**2d**) concentrations from liver biopsies of cows. Data was collected on d - 8, 54, or 77 of the study where d - 8 and 54 had n = 30 and d 77 had n = 11 due to early calving. Significant differences, due to a treatment * day interaction, were detected in hepatic Fe ($P = 0.02$), Cu ($P < 0.01$), Co ($P = 0.05$), and Mn ($P < 0.01$) status. There was no effect due to treatment detected between CON and DDG cow hepatic mineral concentrations. The * denotes differences between means detected at $P \leq 0.05$.

Figure 3 a - c. Hepatic mineral concentrations of cows fed either a control supplement (**CON**) or dried distiller's grains with solubles (**DDG**), n = 3. The data presented are covariate corrected least squares means of Zn (**3a**), Se (**3b**), and Mo (**3c**) concentrations

from liver biopsies of cows. Data was collected on d-8, 54, or 77 of the study where d -8 and 55 had n = 30 and d 77 had n = 11 due to early calving. Neither significant differences due to a treatment * neither day interaction, nor due to treatment was detected between CON and DDG cow hepatic mineral concentrations. The † denotes differences between means detected at $0.05 < P \leq 0.10$.

Figure 1.


1a
640
630
620
610
600
590
580
570
560
550
540
BW, kg
DDG
CON
1b
5.60
5.40
5.20
5.00
4.80
4.60
4.40
4.20
BCS
1c
11.50
10.50
9.50
8.50
7.50
6.50
5.50
BUN, mM
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54
77
Day of Trial

Figure 2.

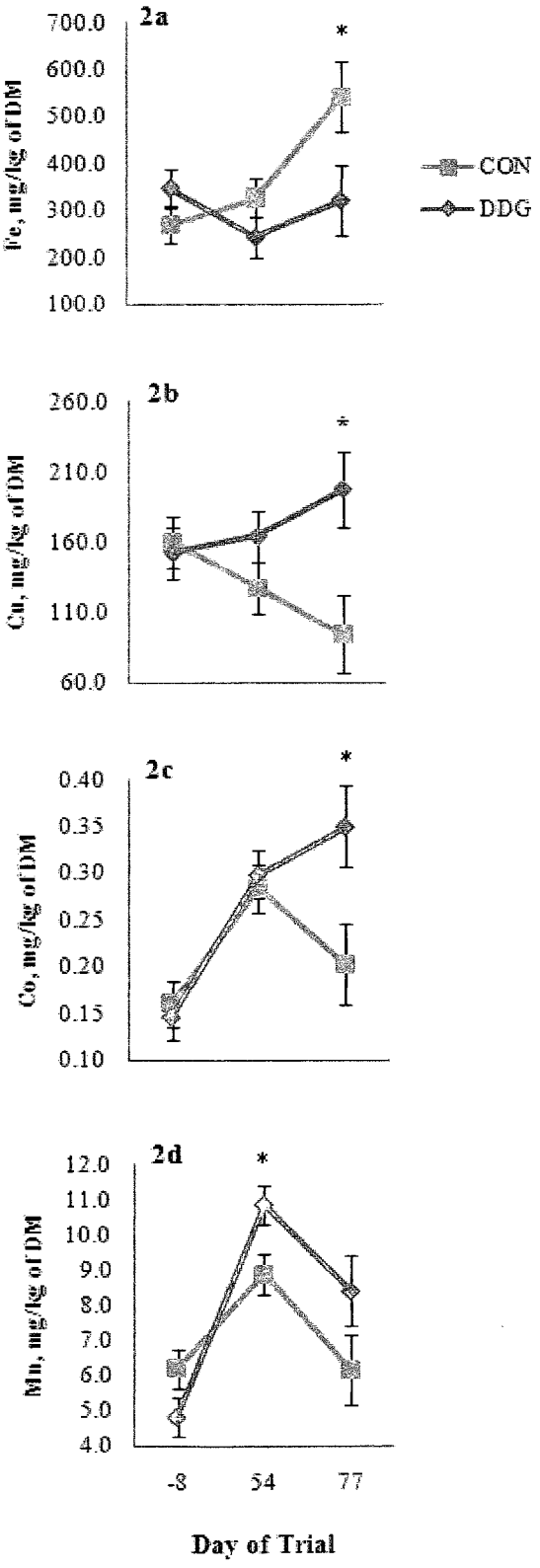
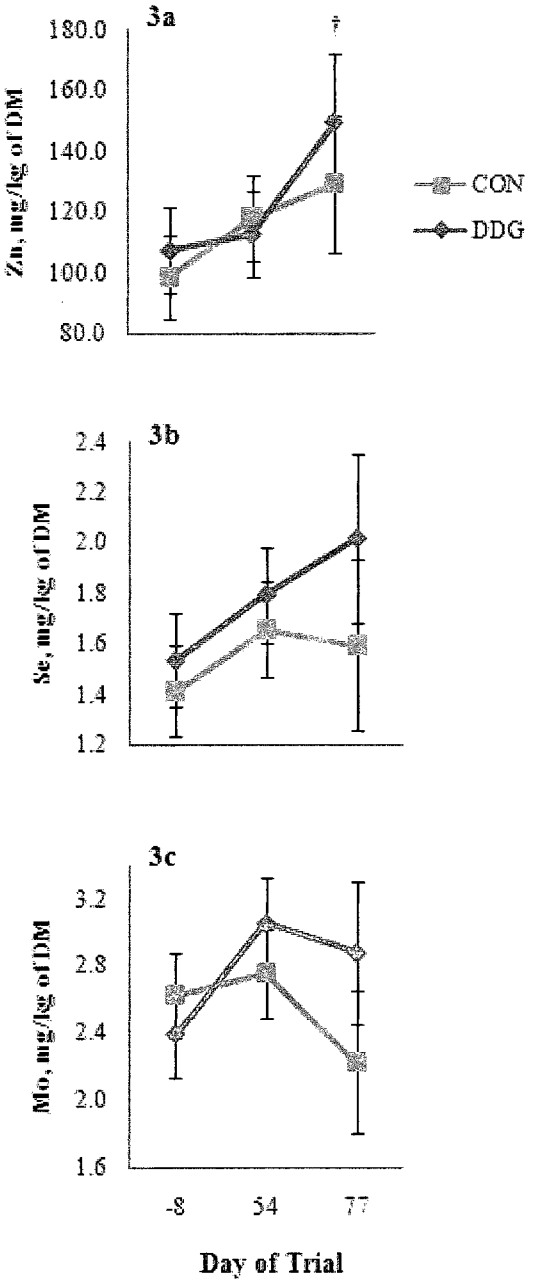

2a
700.0
600.0
500.0
400.0
300.0
200.0
100.0
Fe, mg/kg of DM
*
CON
DDG
2b
260.0
210.0
160.0
110.0
60.0
Cu, mg/kg of DM
*
2c
0.40
0.35
0.30
0.25
0.20
0.15
0.10
Co, mg/kg of DM
*
2d
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
Mn, mg/kg of DM
*
-8
54
77
Day of Trial

Figure 3.


3a
180.0
160.0
140.0
120.0
100.0
80.0
Zn, mg/kg of DM
†
CON
DDG
3b
2.4
2.2
2.0
1.8
1.6
1.4
1.2
Se, mg/kg of DM
3c
3.2
2.8
2.4
2.0
1.6
Mo, mg/kg of DM
-8
54
77
Day of Trial

CHAPTER III

EVALUATION OF SACCHAROMYCES CEREVISIAE FERMENTATION PRODUCT AS A NATURAL ALTERNATIVE TO AN IONOPHORE ON GROWTH PERFORMANCE, COST OF GAIN, AND CARCASS CHARACTERISTICS OF HEAVY-WEIGHT YEARLING BEEF STEERS

Evaluation of *Saccharomyces cerevisiae* fermentation product as a natural alternative to an ionophore on growth performance, cost of gain, and carcass characteristics of heavy-weight yearling beef steers[1,a]

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[a] This manuscript was prepared following Journal of Animal Science style and form.

ABSTRACT: Two-hundred fifty-two cross-bred yearling steers (406 ± 24 kg BW) were used in a completely randomized block design with a 2 x 2 factorial arrangement of treatments (7 pens / treatment) to evaluate the effects of dietary *Saccharomyces cerevisiae* fermentation product (**SFP**) and monensin (**MON**) on growth performance, cost of gain (**COG**), and carcass characteristics. Dietary treatment factors were 1) with or without SFP and 2) with or without MON in finishing diets with 19.7% inclusion of dried distiller's grains with solubles (**DDGS**; DM basis). Both SFP and MON were offered in the total mixed ration in place of an equal amount of cornmeal (DM basis; target intake = 2.8 g and 33 mg / kg of DMI, respectively). Each treatment group was offered ad libitum access to a transition ration from d 1 to 8, then to the finishing ration from d 9 to 125. Rations were formulated to be isonitrogenous and isoenergetic. Steers were not implanted during the study. Body weights were collected on d 0, 28, 56, 84, 110, and 125. Initial and final BW was an average of two-day weights (d -1 and 0; d 124 and 125, respectively). Steers were shipped for harvest on d 125. Overall ADG was decreased ($P \leq 0.028$) in steers supplemented with YC however, final BW was similar among treatments. Feeding YC was associated with a lower ($P = 0.007$) HCW and a greater ($P = 0.003$) number of carcasses grading Choice. Although not significant, YC steers had the highest percentage of yield grade 1 and 2 (81%) and premium Choice and Choice (67%) carcasses. Twelfth rib fat thickness and frequency of USDA yield grade 4 and 5 carcasses tended to decrease when steers were fed YC alone, but tended to increase when steers were fed MON (YC x MON, $P = 0.072$ and $P = 0.086$, respectively). There was no difference due to treatment on COG. These data indicate that feeding YC may improve carcass characteristics of steers finished at lower end weights, which could result

in fewer days on feed. The effects of MON in the current study may have been limited in heavy yearling steers due to consumption of a finishing diet containing 19.7% DDGS.

Key words: beef, carcass, ionophore, performance, *Saccharomyces cerevisiae* fermentation product, yeast culture

INTRODUCTION

It is current conventional practice to administer antimicrobials, such as monensin (**MON**) as a growth-promoting feed additive in finishing beef feedlot diets in the U.S. Monensin, also referred to as an ionophore (Schelling, 1984), is widely used for improved feed efficiency, reduction in digestive upsets, and prevention of coccidiosis (Goodrich et al. 1984). However, there is growing consumer demand for natural and organically-grown beef (Grannis and Thilmany, 2000; Thompson et al., 2007). The USDA (2009) designates that naturally-raised beef animals are to be grown without the use of growth hormones or antimicrobials. Therefore, establishing an economically competitive, natural alternative to ionophores, that fits under the USDA's designation for natural and organically-grown beef, and that does not compromise end-product quality, is of interest to researchers and beef producers. Such an alternative could be a *Saccharomyces cerevisiae* fermentation product (**SFP**), also referred to as yeast culture (**YC**). *Saccharomyces cerevisiae* fermentation products have been shown to stimulate rumen bacterial yield by providing soluble growth factors (Callaway and Martin, 1997), increase mineral retention (Cole et al., 1992) and nutrient digestibility (Wohlt et al., 1991), stabilize ruminal pH, and reduce the incidence of acidosis (Dawson et al., 1990). Comparative effects of YC and ionophores on growth performance and carcass traits are

lacking. Therefore, the objective of this study was to evaluate SFP as a natural alternative to MON on growth performance, cost of gain (**COG**), and carcass characteristics of finishing heavy-weight yearling beef steers.

MATERIALS AND METHODS

Animals

All sampling techniques, animal use, and handling were pre-approved by the Colorado State University Animal Care and Use Committee.

One day before the start of this experiment (June 11th, 2008), 312 cross-bred yearling beef steers were received at the Southeast Colorado Research Center (**SECRC**; Lamar, CO). Prior to entry at SECRC, and before being sorted off as candidates for the study, the cattle were backgrounded for ~30 d and received Revalor® -S (Intervet / Schering-Plough Animal Health Inc., Millsboro, DE), Pyramid® 2 + Type II BVD (Fort Dodge Animal Health, Fort Dodge, IA), ProMectin™ (ivermectin) (IVX Animal Health, Inc., St. Joseph, MO), Presponse SQ® (Fort Dodge Animal Health, Fort Dodge, IA), and Safe-Guard® Suspension 10% (fenbendazole) (Intervet / Schering-Plough Animal Health Inc., Millsboro, DE). Once selected for the study, the steers were ranked and stratified by coat color score, unshrunk BW, and rectal temperatures following in-processing at SECRC. Cattle did not receive any growth implants upon or post-arrival at SECRC. Individuals receiving a coat color score representative of either Brahman or Holstein-influence and that were beyond ± 2 standard deviations from mean unshrunk BW (406 ± 24 kg) or mean rectal temperature (39.6 ± 0.33°C) were eliminated from further consideration for the study.

The remaining 252 eligible steers were blocked by unshrunk BW (7 blocks) and randomly assigned a number from 1 to 1000 using the random number function in Microsoft Excel (Microsoft, Redmond, WA). Steers were then ranked according to weight. For each successive set of 7 steers, the individual with the lowest random number was assigned to replicate one, the second lowest random number assigned to replicate two, and so forth until the individual with the highest random number within the set was assigned to the seventh replicate. This process was repeated for each successive group of steers until all steers had been assigned to one of 7 replicates. By following this randomization schedule, n = 7 replicates of 9 steers of similar weight distribution were assembled for each of the 4 treatments in the study. Following their initial weighing on d 0, steers had access to long-stem grass hay and water overnight. The following morning, steers were reweighed and received visual tags identifying trial, treatment, replicate, and individual steer within trial. Steers were sorted and housed in dirt surfaced pens (6.1 m x 18.3 m) with 3.5 m of linear bunk space located on a concrete feeding apron. Every two pens shared a common water fountain that was located along the fence line. No wind breaks or shade structures were provided.

Diets and DMI

Exposure to dietary treatments was initiated on d 1. Each pen replicate was randomly assigned to 1of 4 dietary treatments factors that were: 1) with or without SFP (Diamond V XP™, Diamond V Mills, Cedar Rapids, IA) and 2) with or without MON (Rumensin®, Elanco, Division of Eli Lilly and Company, Greenfield, IN) in finishing diets containing 19.7% inclusion of dried distiller's grains with solubles (**DDGS**; DM basis). Each treatment group was offered a transition ration from d 1 through 8, and then

was moved to a finishing ration from d 9 through the duration of the trial. Dry matter and chemical composition of each diet is shown in Table 1. All rations during both phases were formulated to be isonitrogenous and isoenergetic. *Saccharomyces cerevisiae* fermentation product was offered at the manufacturer's recommended inclusion level of 56 g animal^{-1}·d^{-1} (as-fed) during the transition phase and then decreased to 28 g animal^{-1}·d^{-1} (as-fed) during the finishing phase (or 2.8 g / kg of DMI). Monensin was offered at the inclusion level of 11 mg / kg of DMI during the transition phase, and then increased to 33 mg / kg of DMI for the finishing phase (Berthiaume et al., 2006). Both SFP and MON were offered in the total mixed ration (**TMR**) in place of an equal amount of cornmeal on a DM basis. Both water and diets were offered ad libitum throughout the study.

Steers were fed twice daily (approximately 0700 and 1700) at an estimated 110% of the previous days ad libitum intake. Diets were mixed in a truck-mounted feed processor immediately prior to feeding. To avoid carryover of SFP and MON from the feed processor to treatment groups not intended to receive SFP or MON, interim TMR for non-study cattle were mixed and fed between batches. Daily deliveries of as-fed TMR were recorded for each treatment pen and used for determination of DMI. Every 7 d, random TMR samples (~ 200 g) from the 0700 feeding were obtained in triplicate. Two of the 3 samples were weighed, dried at 55°C for 48 h, and then reweighed for determination of percent DM (Table 1). Samples were then ground in a Wiley Mill (Thomas Model 4, Swedesboro, NJ), passed through a 1-mm screen, and stored in a sealed, plastic bag at 4°C pending analysis. The third sample was stored (as-fed) at 4°C to serve as a back-up. At the end of each 28-d period, the two DM samples from each

week were mixed with other weekly samples from that 28-d period to make a composite 28-d TMR sample, which was then submitted to a commercial lab (SDK Laboratories, Hutchinson, KS) for analysis of nutrient composition. The fraction of DM was used to calculate delivery of DM to each pen, which was then divided by the number of steers in that pen to arrive at DMI / animal. Feed bunks were cleaned and orts were collected on a weekly basis (before the 0700 feeding), weighed, analyzed for DM content, and subtracted from the original feed offered to determine actual feed intakes.

Performance and Carcass Characteristics

Steer performance was monitored by collection of BW on d 0, 28, 56, 84, 110, and 125. Body weight values recorded during each weigh-day were transformed to shrunk BW (**SBW** = BW * 0.96) for analysis. Initial and final SBW was an average of two-day weights (d -1 and 0; d 124 and 125, respectively). Following each processing and weighing procedure, steers were returned to their designated treatment pens to continue existing ration and treatment assignments through d 125. Determination and calculation of DMI is described above. Average daily gain and feed efficiencies were calculated on a live basis for each 28-d period. Incidence and description of morbidity and mortality were recorded. Production costs were calculated for each treatment group for each 28-d period using this formula: cost / kg of gain = (cost of feed on DM basis) x (feed-to-gain ratio; **F:G**). Steers were shipped for harvest on d 125. Carcass measurements were obtained for all steers by a data collection service (Cattlemen's Carcass Data Service, Canyon, TX) at a commercial slaughter facility. Hot carcass weight and liver abscesses were recorded at slaughter, whereas other carcass

measurements were obtained after a 24-h chill. Marbling scores and USDA quality and yield grades (**YG**) were determined by a USDA grader.

Statistical Analysis

The trial was conducted as a 2 x 2 factorial, completely randomized block design with repeated measures. Pens of steers (or replicates) were treated as the experimental unit and individual steer as the sampling unit in the analysis. Analysis of variance for each continuous response variable was performed using mixed model procedures in Statistical Analysis Software (**SAS**, v9.1.3, Cary, NC). The model included a repeated measures statement, the random effect of block, and the fixed effects of SFP, MON and data collection day, and the interaction thereof. The continuous response variables analyzed by this model included SBW, DMI, ADG, F:G, G:F, COG, HCW, dressing percentage, longissimus muscle area, KPH, 12^{th} rib fat thickness, USDA YG, marbling score, and quality score. In addition to the mixed model, discrete response variables were also analyzed using Glimmix and chi-square frequency procedures in SAS. The discrete response variables analyzed by this model included the frequency (or incidence) of morbidity, mortality, USDA YG, USDA quality grades, and liver abscesses. Either a "1" or "0" was assigned to those response variables that did or did not, respectively, occur for each individual animal or carcass. Where applicable, Tukey's comparison procedure was used to test differences between least squares means if significant (or tendencies of) main effects or interactions were found. Significance was declared at $P \leq 0.05$, and a tendency at $0.05 < P \leq 0.10$.

RESULTS

One steer from the MON treatment was treated and then removed on d 110 for respiratory reasons that resulted in death. This was the only incidence of morbidity or mortality in the study. There were no SFP x MON interactions detected for any growth performance traits (Table 2). When BW was analyzed as a repeated measure (Figure 1), there was a main effect of day on BW ($P < 0.01$), where all cattle gained weight as the study progressed. Additionally, ADG was lower ($P = 0.028$) in steers supplemented with SFP. Although there was no difference ($P > 0.05$) due to treatment, COG was numerically highest for SFP steers ($2.00 / kg of BW gain). Twelfth rib fat thickness and frequency of USDA YG 4 and 5 carcasses tended to decrease when steers were fed SFP alone, but tended to increase when steers were fed MON (SFP x MON, $P = 0.072$ and $P = 0.086$, respectively; Table 3). Feeding SFP was associated with a lower ($P = 0.007$) HCW and a greater ($P = 0.003$) number of carcasses grading Choice (**Ch**). Although not significant, SFP steers had the highest percentage of YG 1 and 2 (81%) and premium Ch and Ch (67%) carcasses.

DISCUSSION

Effect of Saccharomyces cerevisiae Fermentation Product. It has been demonstrated that YC promotes a higher ruminal pH environment by reducing the concentration of L-lactate (Dawson et al., 1990; Williams et al. 1991; Erasmus et al., 1992). This elevated pH would not only reduce the incidence of acidosis (Dawson et al., 1990), but would also be more favorable for the growth of cellulolytic bacterial species, such as *Fibrobactor succinogenes, Ruminococcus albus,* and *Ruminococcus flavefasciens* in the rumen (Callaway and Martin, 1997, Miller-Webster et al., 2002). In a review of

the literature, the most consistent response to YC is stimulation of rumen cellulolytic bacteria. Reports range from 5 to 40 times greater number of cellulolytic species present, particularly in ruminants fed high-roughage diets (Hillman et al., 1985; Newbold et al., 1993). In addition to stabilizing rumen pH, YC stimulates rumen bacterial yield by providing soluble growth factors, such as organic acids, B vitamins, and amino acids (Callaway and Martin, 1997). Enhancing the fermentative capacity of rumen cellulolytic species has been shown to increase fiber digestion, mineral retention, and flow of microbial protein from the rumen (Cole et al., 1992; Martin and Nisbet, 1992; Newbold et al., 1996). Yeast culture supplemented to lactating Holstein cows (fitted with rumen and duodenal cannulas) had higher DM disappearance and digestibilities of CP and ADF (Erasmus et al., 1992). Others have demonstrated increased digestibility, specifically the fiber and protein portion of the diet, when YC was offered (Wohlt et al., 1991; Miller-Webster et al., 2002).

The increased prevalence of the fuel ethanol industry has decreased the availability of corn as an energy source for beef finishing rations, but has been replaced by by-products such as wet and dried distiller's grains (Vasconcelos and Galyean, 2007a). In the current study, DDGS were included in the ration not only due to price competitiveness, but also because DDGS are higher in NE, NDF, and CP concentrations and lower in starch on a DM-basis than corn (NRC, 2000). Although flaked corn remained in the finishing diet at 63.5 % (DM), the inclusion of DDGS lowered the amount of NE_G that would have otherwise needed to come from corn, thereby elevating the proportion of CP and NDF, and decreasing starch concentration in the diet. In a recent survey conducted by Vasconcelos and Galyean (2007b), the majority of beef

finishing rations are formulated to include 70 - 85% grain on a DM basis. The inclusion level of flaked corn in the current study was slightly below those values. Additionally, in an effort to target current industry feeding practices, the inclusion level of DDGS in the current study were based off of figures provided by the Vasconcelos and Galyean (2007b) survey. The survey indicated that 82.76% of nutritionists formulate rations to include 5 – 50% ethanol by-products (DM basis) in finishing rations (average = 16.5%; mode = 20%). Rations in the current study contained roughly 18 and 19.7 % (DM basis) in the transition and finishing rations, respectively.

Considering the aforementioned benefit on CP and NDF digestion with SFP in the diet, a synergistic effect between DDGS and SFP was expected, resulting in an increased feed efficiency and growth in the finishing steers. In disagreement with our hypothesis, SFP-fed cattle did not significantly outperform the CON or MON cattle. The reason for this is unknown. Previously, Cole et al. (1992) reported no affect on growth performance of calves fed increasing levels of SFP in receiving diets. However, when calves were challenged intranasally with infectious bovine rhinotracheitis virus, those calves receiving SFP had higher DMI and improved weight gain. Similarly, Phillips and VonTungeln (1985) reported that the addition of YC in the receiving ration of feeder calves tended to increase DMI, but had no consistent effect on ADG. The authors from both studies concluded that YC supplementation seemed to provide a greater benefit in stressed calves. Additionally, Phillips and VonTungeln noted that when YC was added to receiving rations containing MON, ADG was depressed compared to those that did not receive the combination of YC and MON (0.87 and 1.04 $kg \cdot animal^{-1} \cdot d^{-1}$, respectively). While cattle in this trial were fed a different YC product at a lower dosage, a similar

response was seen in the current study when SFP was added to the MON diet (S x M). Average daily gain was lower than when only MON was included. Conversely, Hinman et al. (1998) reported that the addition of YC in the finishing diet of cross-bred steers increased both ADG and G:F. Schingoethe et al. (2004) reported increased feed efficiency in mid-lactation Holstein cows when 60 g $cow^{-1} \cdot d^{-1}$ YC was offered. Comparatively, in the Schingoethe study, the dosage of YC was slightly higher, as was the percentages of CP and NDF in the diet than in the current study (60 g $animal^{-1} \cdot d^{-1}$, 17.5 %, and 30.8 % versus 56 g $animal^{-1} \cdot d^{-1}$, ~13% and ~18 %, respectively). This disparity between study results could be attributed to differences in dosage of YC, concentration of NDF and starch in the diet, and the level of stress experienced by the animals. In the current study, only 1 steer was treated and removed from the study due to health reasons which indicates that cattle were minimally stressed and generally in good health, which could be the explanation for a lack of growth performance response due to the dietary supplements offered. It also seems likely that because cattle were heavy-weight (406 ± 24 kg BW) yearling steers, that the age and weight of cattle likely contributed to the minimal stress experienced by the cattle when introduced to the finishing study conditions.

Carcass data indicates that SFP-fed steers had (numerically) improved carcass merit compared to CON or MON steers in USDA yield and quality grades, having the highest combined percentage of YG 1 and 2 (81%) and of USDA premium Ch and Ch (67%). It is worth noting that although the carcasses of SFP-fed steers were superior in carcass USDA yield and quality grades, they were also the lightest in HCW. These results could be interpreted to suggest that SFP steers were more optimally finished at a

lower end weight than either the CON or MON-fed steers and may not require as many days on feed. The positive effect of SFP on quality grade has been previously reported (unpublished, Diamond V Mills, 1993). However, the difference in USDA quality grade is not supported by a corresponding difference in either quality or marbling score. It is likely that this discrepancy could be attributed to how similar the carcasses were within the Ch / Select (**Se**) USDA grading assignment. The National Beef Quality Audit (Garcia et al., 2008) reported that 79.96% of carcasses (≥ USDA YG 1) from US fed steers and heifers fall within the Ch / Se USDA quality grading assignment, and that the vast majority of the marbling scores are in the lower grade levels (e.g. low Ch = 64.21%).

An increase in total VFA production and a decrease in ruminal acetate : propionate concentrations has been previously reported when SFP was offered to ruminants (Williams et al., 1991; Carro et al., 1992; Erasmus et al., 1992). Although not measured, the authors hypothesize that YC supplementation may have caused an increase in total ruminal VFA production along with a decreased acetate:propionate ratio as compared to CON and MON-fed cattle. Increased VFA production, particularly concentration of propionate, could potentially increase intramuscular fat deposition and be the explanation for the increased number of carcasses that graded Ch or higher from SFP-fed steers. Smith and Crouse (1984) reported that acetate will provide 70 – 80% of the acetyl units to *in vitro* lipogenesis in subcutaneous adipose tissue and only 10 – 25% in intramuscular adipose tissue. Conversely, glucose (made from propionate in the liver) will provide 1–10% of the acetyl units in subcutaneous adipose tissue and 50–75% in the intramuscular depot. Therefore, feeding SFP could alter VFA concentrations in such a way that would positively affect marbling and carcass quality.

Effect of Monensin. Feeding MON had no effect on growth or carcass characteristics. Although our carcass results are consistent with previous studies, growth performance results are conflicting. Multiple studies on feeding MON to ruminants have been consolidated (Goodrich et al., 1984; Nagaraja et al., 1997) and indicate that across various diets, types of cattle, and conditions, MON has consistently improved feed efficiency, reduced feed intake, lactic acid production, and the likelihood of bloat, and decreased the incidence of coccidiosis. Perhaps the disparity in growth performance between the current and previous studies could be explained by the mode of action of MON relative to the ingredient composition of the TMR.

Ionophores modify the movement of ions across biological membranes, specifically causing Na entry into cells (Haney and Hoehn, 1967; Pressman, 1976; Smith and Rosengurt, 1978). The biological response of this action has been previously outlined (Schelling, 1984; Nagaraja et al., 1997). In causing this flux of ions, MON increases the molar proportion of propionate at the expense of lactate and concurrently decreases molar proportions of acetate and butyrate produced in the rumen. An increase in propionate improves energy utilization of MON-fed animals. Consequently, these changes in ruminal fermentation, caused by feeding MON, have been shown to prevent acidosis and bloat. Typically, MON is fed in diets high in rapidly fermentable carbohydrates as a preventative measure against such digestive disturbances. In the current study, the authors decreased the proportion of rapidly fermentable, high-concentrate feedstuffs with DDGS. As already mentioned, DDGS are higher in NDF and lower in starch on a DM-basis than corn (NRC, 2000). Nagaraja et al. (1997) reported moderate to marked inhibition of fiber digestibility when cattle were fed MON. Perhaps

the discrepancy between the lack of effect on growth performance in MON-fed steers compared to previous studies could be explained by the inability of MON to be effective when fed in TMR containing distiller's grains. Although the inclusion of corn was still relatively high in our study, it was still below industry averages (Vasconcelos and Galyean, 2007b). It seems possible that an effect on growth performance due to MON was not detected because some of the starch was replaced with fat by DDGS; hence, the mode of action of MON may have been limited. Recently, Depenbusch et al. (2008) reported that feeding MON to finishing heifers offered no growth performance or carcass advantage when distiller's grains replaced a portion (25%) of the steam-flaked corn in the TMR. Meyer et al. (2009) reported no significant advantage in ADG or carcass traits of steers fed finishing diets containing distiller's grains and supplemented with MON. However, contrary to the current study where cattle fed MON had no effect on F:G, Meyer et al. (2009) reported a significant improvement in F:G when MON was fed to cattle consuming 25% wet distiller's grains and 29.75% each of high-moisture corn and dry-rolled corn.

Although feeding MON had no significant effect on carcass characteristics, cattle fed MON had numerically the highest HCW, incidence of liver abscesses, and number of carcasses to grade select or lower, and the numerically the lowest USDA quality and yield grades. Carcass results are in agreement with 228 previous trials, summarized by Goodrich et al. (1984), which involved 11,274 head of cattle fed MON-containing diets. The summary indicated that dressing percentage, marbling score, fat depth, quality grade, and yield grade were either not affected or negatively affected by MON. Additionally,

the inclusion of MON has shown no effect on liver abscess incidence in several studies previously summarized by Nagaraja and Chengappa (1998).

Production Costs. The average price for each of the rations (on a DM-basis) was as follows: CON = $12.61, SFP = $12.59, MON = $12.51 and S x M = $12.66 / cwt of DM (data not shown). The explanation for the price of the CON ration being higher than either the SFP or MON rations is due to the average DM of the CON ration was the lowest, therefore increasing the price on a DM-basis. However, when price / cwt is converted to price / kg of DM, all TMR were = $0.28 / kg of DM (data not shown). Therefore, with ration costs being equal on a per kg of DM basis, differences in cost / kg of gain is determined solely on feed efficiency where COG = (cost of feed / kg on DM basis) x (F:G ratio). Although the COG was highest for the SFP-fed cattle, the difference was not statistically significant. This value is highest due to the highest numerical value for F:G in the SFP-fed steers. Cattle fed SFP cost approximately 5.82% more to feed than MON-fed cattle. Previous work shows that naturally-fed cattle tend to cost 39% more (Fernandez and Woodward, 1999) and that consumers are willing to pay more for the product (Boland et al., 2002). Based on this study, the producer would need to be compensated by a market premium of at least 6% to maintain their profit margin per kilogram of gain if SFP was used as a natural alternative to MON for the production of "natural beef". However, it could be elucidated, due to a higher percentage of carcasses from SFP-fed cattle that graded USDA YG 1 or 2 and quality grade Ch or better, that cattle could be finished a lower end weights. This would allow for fewer days on feed and lower total production costs.

Lastly, it could be argued that the implanting that occurred during the backgrounding stage (before the cattle were selected to be in this study) may have impacted the results in such a manner that differences due to treatment could not be detected in some cases. Previous research (Guiroy et al., 2002) indicates no difference in ADG or G:F when cattle are implanted with either Revalor®-S (**Rev-S**; formulated for steers) or Revalor®-H (formulated for heifers) in a single, initial implant strategy compared to receiving a second, re-implant (64 – 71 d following initial implant) with the same product. This same study also indicates an increase in ADG and G:F in implanted (single or double) cattle compared to controls receiving no anabolic implant strategy. Additionally, Roeber et al. (2000) reported no difference in HCW, KPH, YG, marbling score, or quality grade between carcasses of cattle implanted with Rev-S in a single initial implant strategy compared to receiving a second, re-implant (59 d following initial implant) with the same product. This same study also indicates an increase in HCW, KPH, and marbling score in carcasses from implanted (single or double) cattle compared to controls receiving no anabolic implant strategy. Lastly, in a study with a similar duration and implant regimen to the current trial, Bruns et al. (2005) indicated that cattle receiving an early Rev-S implant on d 1 of a 140-d trial had increased G:F, but otherwise no difference in BW, ADG, or DMI to cattle receiving no implants. It seems likely, that had the cattle in the current study not received an implant prior to the onset of treatments, that the growth performance and carcass merit of the cattle would have likely been different.

Summary and Implications. In conclusion, there seems to be some benefit, particularly from a carcass merit stand-point, from feeding SFP. The results of this study

indicate that the response of feedlot cattle to MON was limited in steers consuming a diet with 19.7% DDGS (DM basis). Therefore, because few differences were detected due to either SFP or MON treatment, the results of the current study indicate that the conditions were favorable for "naturally-fed" cattle to perform well without any feed additive. What may have been novel in this study was good bunk management, ration design, weather, and a subset of cattle that would allow for abandonment of feed additives. Admittedly, although the finishing phase of this trial was designed to emulate natural-beef feeding practices for the CON and SFP group, it would have been ideal for the steers to have not received implants before the trial. Regardless, the authors feel that further research is needed for determination of effective dosage level of SFP in finishing beef rations dependent upon varying levels of roughage and starch. Feeding SFP may render more benefit in growth performance if fed at a higher dosage level or when fed to cattle under different feeding and management strategies.

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TABLES AND FIGURES

Table 1. Dry matter and chemical composition of diets[1]

	Transition[2]				Finishing[3]			
Item	CON	SFP	MON	S x M	CON	SFP	MON	S x M
Ingredient, % of TMR[4]								
Alfalfa Hay	9.3	9.3	9.3	9.3	5.5	5.5	5.5	5.5
Corn silage	12.6	13.2	12.6	13.2	9.0	9.0	9.0	9.0
Flaked Corn	57.8	56.9	57.7	56.9	63.5	63.5	63.5	63.5
DDGS[5]	18.1	18.3	18.1	18.3	19.7	19.7	19.7	19.7
Limestone	1.5	1.5	1.5	1.5	1.6	1.6	1.6	1.6
Corn Meal[6]	0.49	0.01	0.48	-	0.31	0.01	0.31	-
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Oil	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Trace mineral premix[7]	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Vitamin E	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016
Vitamin A	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
Yeast Culture[8]	-	0.526	-	0.526	-	0.311	-	0.311
Monesin[9]	-	-	0.015	0.015	-	-	0.016	0.016
Nutritional composition[10]								
DM, %	71.28	71.49	73.38	73.36	74.75	74.76	75.52	74.85
CP, %	12.75	13.18	13.28	12.78	14.09	13.90	13.96	13.77
NPN, %	0.40	0.31	0.26	0.29	0.61	0.40	0.33	0.35
NDF, %	17.98	18.08	17.50	18.16	16.78	16.55	17.15	17.37
Ether extract, %	5.28	5.35	5.16	5.05	5.70	5.41	5.66	5.71
NE_G, Mcal/kg[11]	1.42	1.42	1.42	1.42	1.47	1.47	1.47	1.47
Calcium, %	0.72	0.66	0.59	0.65	0.82	0.80	0.83	0.73
Phosphorus, %	0.39	0.40	0.40	0.38	0.39	0.39	0.39	0.38
Potassium, %	0.76	0.75	0.73	0.75	0.74	0.74	0.79	0.72
Magnesium, %	0.21	0.21	0.19	0.20	0.19	0.18	0.18	0.17
Sulfur, %	0.26	0.30	0.27	0.28	0.24	0.26	0.26	0.27

[1]Diets consisted of a basal control (**CON**) diet, supplemented with or without *Saccharomyces cerevisiae* fermentation product (**SFP**; Diamond V® "XP", Diamond V Mills, Inc., Cedar Rapids, IA) and with or without an ionophore (**MON**; Rumensin®, Elanco, Division of Eli Lilly and Company, Greenfield, IN), and the two-way interaction of SFP x MON (**S x M**).

[2]Transition ration fed for d 0 - 8 of trial.

[3]Finishing ration fed for d 9 - 125 of trial.

[4]Ingredients are partitioned as percentage of total mixed ration (**TMR**) on a DM-basis.

[5]DDGS = dried distiller's grains.

[6]Both the SFP and MON were offered in the total mixed ration place of an equal amount of cornmeal on a dry-matter basis.

[7]Formulated to provide the following on a DM basis: 0.70% Ca, 0.39% P, 0.78% K, 0.25% Mg, 0.25% S, 0.25% NaCl, 75 mg/kg Zn, 85 mg/kg Fe, 10 mg/kg Cu, 25 mg/kg Mn, 0.20 mg/kg Co, 0.25 mg/kg I, and 0.12 mg/kg Se.

[8]SFP was offered at manufacturer's recommended inclusion rate of 56 g animal^{-1}·d^{-1} during transition phase and decreased to 28 g animal^{-1}·d^{-1} during finishing phase (2.8 g / kg of DMI).

[9]The ionophore was offered at the inclusion rate of 11 mg / kg of DMI during transition phase and increased to 33 mg / kg of DMI during finishing phase, as previously described by Berthiaume et al. (2006).

[10]With the exception of NE_G, values reported for nutritional composition of diets are based off of nutrient analysis from TMR samples taken every 7 d throughout the study. Values reported are a percentage of DM.

[11]Rations were formulated for NE_G values listed, which was calculated based off of NRC (2000) values.

Table 2. Finishing performance of pens of steers fed diets supplemented with or without *Saccharomyces cerevisiae* fermentation product and with or without an ionophore (n = 7)[1]

	Treatment					P-value		
Item	CON	SFP	MON	S x M	SE	SFP	MON	S x M
Number of steers[2]	63	63	62	63	-	-	-	-
Number of pens	7	7	7	7	-	-	-	-
Performance								
Initial BW[3], kg	391.1	391.9	392.9	391.3	8.07	0.956	0.944	0.885
Final BW[3], kg	603.0	590.1	603.0	592.3	7.91	0.109	0.877	0.882
DMI, kg	10.38	10.17	10.43	9.72	0.30	0.122	0.487	0.385
ADG, kg	1.679[a]	1.544[b]	1.616[a]	1.574[b]	0.04	0.028	0.677	0.238
G:F	0.165	0.154	0.157	0.165	0.01	0.823	0.771	0.142
F:G[4]	6.50	7.32	6.95	6.70	0.40	0.464	0.833	0.181
COG[5]	1.77	2.00	1.89	1.85	0.11	0.407	0.916	0.209
Morbidity[6], %	0	0	2	0	-	1.000	0.318	1.000
Mortality[6], %	0	0	2	0	-	1.000	0.318	1.000

[1]Diets consisted of a basal control (**CON**) diet, supplemented with or without *Saccharomyces cerevisiae* fermentation product (**SFP**; Diamond V® "XP", Diamond V Mills, Inc., Cedar Rapids, IA) and with or without an ionophore (**MON**; Rumensin®, Elanco, Division of Eli Lilly and Company, Greenfield, IN), and the two-way interaction of yeast x ionophore (**S x M**).

[2]On d 110 of the trial, 1 steer was removed from the study for respiratory problems that lead to mortality.

[3]The BW values are reported as shrunk BW (**SBW** = BW * 0.96). Initial BW was based on an average of both d -1 and 0 values, interim BW's were collected on days 28, 56, 84, 110, and then final BW was based on an average of both d 124 and 125. Steers were shipped for harvest on d 125 of the study.

[4]Feed-to-gain ratio (**F:G**)

[5]Cost / kg of gain (**COG**) = (cost of feed on DM basis) x (F:G).

[6]Data analyzed using Chi-square test; no SEM available.

[a,b]Least squares means without a common superscript differ, $P \leq 0.05$.

Table 3. Carcass characteristics of pens of steers fed diets supplemented with or without *Saccharomyces cerevisiae* fermentation product and with or without an ionophore (n = 7)[1]

	Treatment					P-value		
Item	CON	SFP	MON	S x M	SE	SFP	MON	S x M
Number of Steers[2]	63	63	62	61	-	-	-	-
Number of Pens	7	7	7	7	-	-	-	-
Carcass traits								
Hot carcass wt, kg	373.5[a]	363.3[b]	375.3[a]	368.3[b]	5.11	0.007	0.278	0.613
Dressing percentage	61.97	62.27	61.60	62.14	0.35	0.494	0.250	0.743
Longissimus muscle area, cm^2	92.2	89.1	92.2	91.5	1.54	0.216	0.454	0.435
Kidney, pelvic, and heart fat, %	1.855	1.896	1.857	1.890	0.05	0.421	0.945	0.922
12-th rib fat, cm	1.176	1.102	1.047	1.142	0.05	0.820	0.349	0.072
USDA yield grade, calculated	2.59	2.58	2.46	2.55	0.10	0.647	0.340	0.593
USDA yield grade 1,[3] %	18	15	5	5	-	0.860	0.514	0.831
USDA yield grade 2,[3] %	58	66	62	56	-	0.869	0.703	0.540
USDA yield grade 3,[3] %	23	19	18	19	-	0.756	0.685	0.652
USDA yield grade 4 and 5,[3] %	2	0	0	5	-	0.386	0.386	0.086
Marbling score[4]	SM^{11}	SM^{22}	SM^{08}	SM^{07}	10.48	0.635	0.357	0.515
Quality score[5]	388	398	387	391	6	0.229	0.418	0.617
USDA Premium Choice,[3] %	10	12	15	3	-	0.239	0.659	0.155
USDA Choice,[3] %	45[b]	55[a]	30[b]	58[a]	-	0.003	0.364	0.152
USDA Select,[3] %	45	33	53	38	-	0.119	0.393	0.782
USDA Standard,[3] %	0	0	2	0	-	0.328	0.328	0.328
Liver abscesses,[3] %	18	23	31	20	-	0.603	0.363	0.115

[1]Diets consisted of a basal control (**CON**) diet, supplemented with or without yeast culture (**SFP**; Diamond V® "XP", Diamond V Mills, Inc., Cedar Rapids, IA) and with or without an ionophore (**MON**; Rumensin®, Elanco, Division of Eli Lilly and Company, Greenfield, IN), and the two-way interaction of yeast x ionophore (**S x M**).

[2]On d 110 of the trial, 1 steer (From SFP group) was removed from the study for respiratory problems that lead to mortality. Also, 2 carcasses (from the S x M group) were not accounted for from carcass data collection service.

[3]Data analyzed using Chi-square test; no SEM available.

[4]SM = Small = 400.

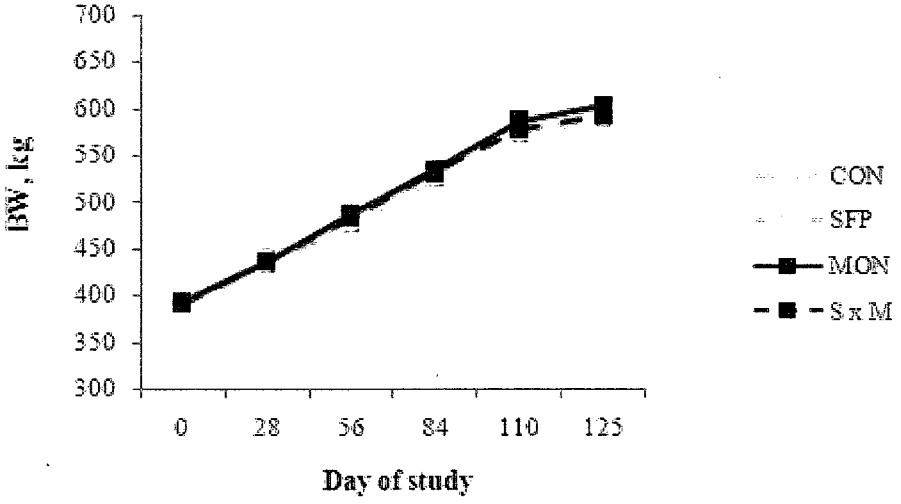
[5]Select = 300-399.

[a,b]Least squares means without a common superscript differ, $P \leq 0.05$.

Figure Legends

Figure 1. Average BW of pens of steers throughout the 125-d trial (n = 7). Body weight values reported are adjusted by removal of 4% shrink and were analyzed as repeated measures. Diets consisted of a basal control (**CON**) diet, supplemented with or without *Saccharomyces cerevisiae* fermentation product (**SFP**; Diamond V® "XP", Diamond V Mills, Inc., Cedar Rapids, IA) and with or without an ionophore (**MON**; Rumensin®, Elanco, Division of Eli Lilly and Company, Greenfield, IN), and the two-way interaction of SFP x MON (**S x M**). There was a main effect of day on BW ($P < 0.01$), where all cattle gained weight as the study progressed. Standard error bars are attached to means, but are not apparent if smaller than the magnitude of the symbol.

Figure 1.


700
650
600
550
500
450
400
350
300
BW, kg
0
28
56
84
110
125
Day of study
CON
SFP
MON
S x M

CHAPTER IV

FECAL PREVALENCE OF *ESCHERICHIA COLI* O157 FROM NATURALLY INFECTED FEEDLOT CATTLE FED COMBINATIONS OF YEAST CULTURE AND AN IONOPHORE IN FINISHING DIETS CONTAINING DRIED DISTILLERS GRAINS WITH SOLUBLES

Fecal prevalence of *Escherichia coli* 0157 from naturally-infected feedlot cattle fed combinations of yeast culture and an ionophore in finishing diets containing dried distillers grains with solubles[a]

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ABSTRACT: Two-hundred fifty-two cross-bred beef steers (BW = 406 ± 24 kg) were used to evaluate the effects of dietary yeast culture (**YC**) and monensin (**MON**) on fecal prevalence of *Escherichia coli* O157:H7 (n = 7 / treatment). Dietary treatment factors were 1) with or without YC and 2) with or without MON in finishing diets with 19.7% inclusion of dried distiller's grains with solubles (**DDGS**; DM basis). The YC and MON were offered for target intake of 2.8 g and 33 mg / kg of DMI, respectively. Environmental samples from the pen floor, feed bunk, and water trough were collected prior to introduction of cattle on d 0. Fecal samples were collected by rectal palpation on d 0, 28, 56, 84, 110, and 125. All samples underwent immunomagnetic separation and were plated onto soribitol MaConkeys agar with novobiocin and potassium tellurite. Further confirmation of isolates as *E. coli* O157:H7 was done using PCR determination for the virulence genes *eaeA*, $fliC_{h7}$, *hly*A, *rfb*, *stx*-I and *stx*-II and were further characterized using PFGE. On d 0, *E. coli* O157:H7 was present in 7.0 % of feed bunk, 14.3 % of pen floor, and 0 % of water trough samples. Cumulative prevalence of the pathogen was 71.4 % in the feces of cattle on d 0 and then decreased ($P < 0.01$) over time. While there was no difference ($P > 0.05$) in fecal prevalence due to dietary treatment, YC-fed cattle presumptively had 0 % prevalence of the pathogen by d 28. Eight unique *Xba*I-PFGE subtype patterns were identified where a predominant subtype (plus 3 closely related subtypes) accounted for 78.7 % of all isolates characterized in the study and were observed in feces and environmental samples on d 0. There was no evidence to support that DDGS stimulated proliferation and shedding of the pathogen in the current study. The results of this study indicate that the feeding of YC to cattle may

decrease, but not eliminate, fecal shedding of *E. coli* O157:H7 upon initial application of the feed additive.

Key words: beef, dried distiller's grains, *Escherichia coli* O157, fecal prevalence, ionophore, yeast culture

INTRODUCTION

The gastrointestinal tracts of beef cattle are recognized as a reservoir for the foodborne pathogen, Shiga toxin-producing *Escherichia coli* O157:H7, which has been implicated with human death, hospitalizations, and disease (Callaway et al., 2003). Beef carcasses can become contaminated with pathogenic *E. coli* due to direct contact with contaminated fecal matter during harvest (Elder et al., 2000). Recently, a positive association between feeding distiller's grains, a co-product of ethanol production, and the fecal prevalence of *E. coli* O157 has been reported (Jacob et al., 2008a, 2008b, 2008c). With approximately 83 % of beef nutritionists indicating that they are currently formulating beef finishing diets to include ethanol co-products (Vasconcelos and Galyean, 2007), these findings have important ramifications for food safety. Therefore, strategies that are effective at reducing pathogen load at the preharvest stage are needed.

Monensin (**MON**), a biologically active compound, often referred to as an "ionophore" for its ability to transport cations across cellular membranes, is routinely fed for growth-promotion and antibiotic activity against coccidiosis in growing and finishing beef cattle (Schelling, 1984). However, ionophores have shown to have either no effect or exacerbate the prevalence of *E. coli* 0157:H7 in the feces of ruminants (Herriott et al.,

1998; Edrington et al., 2003). Additionally, there is growing public concern of antibiotic resistance (PCIFAP, 2006) and consumer demand for naturally-raised beef (Grannis and Thilmany, 2000; Thompson et al., 2007), where the use of ionophores for growth promotion is not permitted (USDA, 2009). Therefore, the subtherapeutic use of antibiotics will likely become more closely regulated; consequently, natural alternatives for growth promotion that may also be effective at reducing pathogen load during the preharvest stage needs to be explored.

A candidate for natural intervention could be dietary supplementation of yeast culture (**YC**). Yeast culture, typically commercially available as *Saccharomyces cerevisiae* fermentation product, can be offered either as, 'a dry product composed of yeast and the media on which it was grown and dried in such a manner as to preserve the fermenting capacity of the yeast' (AAFCO, 2007), or as a direct-fed microbial (**DFM**) only if the YC contains 'live (viable) yeast cells,' (FDA, 1995). The former would better fit the category of a protein supplement or a *prebiotic*. *Prebiotics* are considered 'a non-digestible food ingredient (typically a carbohydrate that can be neither hydrolyzed nor absorbed by the upper part of the digestive tract) that beneficially affects the host by stimulating growth or activity of the natural flora' (Gibson and Roberfroid, 1995). In a recent review, Callaway et al. (2003) hypothesized that prebiotics may provide the native microflora with a competitive advantage against the exclusion and displacement of pathogenic bacteria in the gastrointestinal tract. Comparative effects of YC and ionophores on fecal prevelance of *E. coli* O157:H7 is lacking. The objective of this study was to evaluate the effect of YC and MON on the prevalence of *E. coli* O157:H7 in feces

of feedlot cattle fed finishing diets with inclusion of dried distiller's grains with solubles (**DDGS**).

MATERIALS AND METHODS

Animals and Study Design

All sampling techniques, animal use, and handling were pre-approved by the Colorado State University Animal Care and Use Committee.

One day before the start of this experiment (June 11th, 2008), 312 cross-bred yearling beef steers were received at the Southeast Colorado Research Center (**SECRC**; Lamar, CO). The month prior to entry at SECRC, and before being sorted off as candidates for the study, the cattle had received Revalor® -S (Intervet / Schering-Plough Animal Health Inc., Millsboro, DE), Pyramid® 2 + Type II BVD (Fort Dodge Animal Health, Fort Dodge, IA), ProMectin™ (ivermectin) (IVX Animal Health, Inc., St. Joseph, MO), Presponse SQ® (Fort Dodge Animal Health, Fort Dodge, IA), and Safe-Guard® Suspension 10% (fenbendazole) (Intervet / Schering-Plough Animal Health Inc., Millsboro, DE). Once selected for the study, the steers were ranked and stratified by coat color score, unshrunk BW, and rectal temperatures following in-processing at SECRC. These cattle did not receive any growth implants upon or post-arrival at SECRC. Individuals that were assigned a coat color score representative of either Brahman-influence or Holstein-influence, were beyond ± 2 standard deviations from mean unshrunk BW (406 ± 24 kg), or mean rectal temperature (39.6 ± 0.33 °C) were eliminated from further consideration for the study.

The remaining 252 eligible steers were blocked by unshrunk BW (7 BW blocks) and then randomly assigned to one of 7 replicates. Following their initial weighing on d 0, steers had access to long-stem grass hay and water overnight. The following morning, steers were returned to the chute, weighed, and visual tags identifying trial, treatment, replicate, and individual steer within trial were applied. Steers were sorted and housed in dirt surfaced pens (6.1 m x 18.3 m) with 3.5 m of linear bunk space located on a concrete feeding apron. Every two pens shared a common water fountain that was located along the fence line. No wind breaks or shade structures were provided.

Diets and Treatments

Exposure to dietary treatments was initiated on d 1. Each pen replicate was randomly assigned to 1of 4 dietary treatments factors that were: 1) with or without YC (Diamond V XP™, Diamond V Mills, Inc., Cedar Rapids, IA) and 2) with or without MON (Rumensin®, Elanco, Division of Eli Lilly and Company, Greenfield, IN) in finishing diets with 19.7% inclusion of dried distiller's grains with solubles (**DDGS**; DM basis). Each treatment group was offered a transition ration from d 1 through 8, and then was moved to a finishing ration from d 9 through the duration of the trial. The DM and chemical composition of each diet is shown on Table 1. All rations during both phases were formulated to be isonitrogenous and isoenergetic. The YC was offered at the manufacturer's recommended inclusion level of 56 g animal^{-1}·d^{-1} (as-fed) during the transition phase and then decreased to 28 g animal^{-1}·d^{-1} (as-fed) during the finishing phase (or 2.8 g / kg of DMI). The MON was offered at the inclusion level of 11 mg / kg of DMI during the transition phase, and then increased to 33 mg / kg of DMI for the finishing phase (Berthiaume et al., 2006). Both the YC and MON were offered in the

total mixed ration in place of an equal amount of cornmeal on a DM basis. Both water and diets were offered ad libitum throughout the study.

Steers were fed twice daily (approximately 0700 and 1700) at an estimated 110% of the previous day ad libitum intake. Diets were mixed in a truck-mounted feed processor immediately prior to feeding. To avoid carryover of YC and MON from the feed processor to treatment groups not intended to receive YC or MON, interim TMRs for non-study cattle were mixed and fed between batches. Every 7 d, random TMR samples from the 0700 feeding were obtained in triplicate. Two of the 3 samples were weighed, dried at 55 °C for 48 h, and then reweighed for determination of % DM (Table 1). Samples were then ground in a Wiley Mill (Thomas Model 4, Swedesboro, NJ), passed through a 1-mm screen, and then stored in a sealed, plastic bag at 4 °C pending analysis. The third sample was stored (as-fed) at 4 °C to serve as a back-up. At the end of each 28-d period, the two DM samples from each week were mixed with other weekly samples from that 28-d period to make a composite 28-d TMR sample, which was then submitted to a commercial lab (SDK Laboratories, Hutchinson, KS) for analysis of nutrient composition. The fraction of DM was used to calculate delivery of DM to each pen, which was then divided by the number of steers in that pen to arrive at DMI / animal. Feed bunks were cleaned and orts were collected on a weekly basis (before the 0700 feeding), weighed, analyzed for DM content, and subtracted from the original feed offered to determine actual feed intakes.

Sampling Procedure

Fecal sampling of cattle was conducted on d 0, 28, 56, 84, 110, and 125. Every animal was processed through conventional processing facilities and restrained in a

hydraulic chute. Approximately 50 g of feces was collected from each animal via rectal palpation utilizing a new set of sterile polythene shoulder-length gloves between collections of subsequent animals. Feces were transferred to a sterile 710 ml Whirl-Pak® bag (Nasco, Modesto, CA) which was placed in a cooler with ice packs before being transported to the laboratory at CSU for processing. Fecal samples remained on ice packs for no more than 8 hrs before being stored over night at 4 °C. Following each fecal collection, steers were returned to their designated treatment pens to continue existing ration and treatment assignments through d 125.

Environmental samples were collected on d 0 from each treatment pen floor, feed bunk, and automatic water trough. Pen floor and feed bunk samples were collected using sterile *sponges*, hydrated with 10 ml buffered peptone water (*BioPro Enviro-Sponge* Bags, International BioProducts Inc., Redmond, WA). Sponges were vigorously passed over the sample site surface and then placed into the sterile bag. Pen floor samples were taken from a 60 x 60 cm space immediately next to the automatic water trough in each pen. Approximately 300 ml of water was taken from each water trough and placed in a sterile 710 ml Whirl-Pak® bag (Nasco). All environmental samples were placed in a cooler with ice packs before being transported to the laboratory at CSU for processing. Environmental samples remained on ice packs for no more than 8 hrs before being stored over night at 4 °C.

Fecal E. coli O157:H7 Analysis

The following morning of each fecal sample collection, fecal samples were individually weighed and a 10 – g subsample of feces was removed from each 50 g fecal sample using a sterilized wood tongue depressor (Fischerbrand, Fischer Scientific,

Houston, TX). The 10 – g subsample was combined with subsamples from the other 8 animals within each treatment pen to create a 90 - g pen sample. Pen fecal samples were placed in 1,627 ml Filter-Pak bags (Nasco) with 810 ml (10:1 dilution) of phosphate buffered tryptic soy broth (TSB - PO_4, Becton, Dickinson and Company, Sparks, MD) as previously described (Barkocy-Gallagher et al., 2005). Fecal slurries were homogenized by hand massaging and incubated for 2 h at room temperature (25 ± 2 °C), followed immediately by 6 h incubation at 42 °C. Following incubation, fecal slurries were stored up to 12 h at 4 °C for pending analysis of immunomagnetic bead separation (**IMS**) using Pathatrix protocol (Matrix MicroScience, Inc, Golden, CO). The fecal slurry bags were placed in the Pathatrix's warming pots (preheated to 37 °C), then the Pathatrix apparatus was inserted into the filtered side of each fecal slurry bag, and then 50 µl of anti-O157 immunomagnetic beads (Dynabeads®, Invitrogen, Oslo, Norway) was added to the connector tubing. The samples were circulated for 60 min at 37 °C, and then the beads were washed with phosphate buffered saline (**PBS**). Following IMS, 50 µl aliquots of the beads + PBS were plated in duplicate onto Soribitol MaConkeys agar (Becton) supplemented with 20 mg/L of novobiocin and 2.5 mg/L of potassium tellurite (**mSMAC**). The mSMAC plates were incubated for 24 ± 2 h at 37 °C.

Environmental samples were prepared differently. Water trough samples were filtered to remove particulate matter using sterile 150 ml Corning bottles with 0.45 µm cellulose acetate low protein binding membrane filters (Corning Inc., Corning, NY). The sponges from the pen floor and feed trough samples, and the filters from the water samples were each placed in 710 ml Filter-Pak bags (Nasco) with 10 ml of phosphate buffered tryptic soy broth. Following incubation as described above, pen floor and feed

trough samples were stored at 4 °C until they are subjected to IMS. The IMS procedure for environmental samples was conducted as described by Barkocy-Gallagher et al. (2002) using anti-O157 immunomagnetic beads (Dynabeads®, Invitrogen, Oslo, Norway). Following IMS, 50 µl aliquots of environmental samples were plated on mSMAC plates as described above.

After incubation, up to 3 colonies displaying *E. coli* O157:H7 morphology were selected from each plate and confirmed to be *E. coli* O157:H7 using a multiplex polymerase chain reaction (**PCR**) assay as previously described by Hu et al. (1999). The PCR was performed in a 96-well format with a volume of 25 µl containing 3.18 µl of a primer solution containing the following: *eaeA* (encodes intimin), $fliC_{h7}$ (encodes the H7 antigen), *hly*A (encodes hemolysin A), *rfb* (encodes the O157 antigen), *stx*-I (encodes Shiga toxin I), and *stx*-II (encodes Shiga toxin II), plus 12.5 µl Go Taq Green (Promega, Madison, WI), 8.08 µl of nuclease-free water, and 1 – 2 µl of DNA template. Each PCR consisted of 20 cycles (2 min at 94 °C; 30 s at 94 °C; 1 min at 59°C and 1 min at 72°C) and 20 additional cycles with annealing temperature (7 min for 72 °C) in a thermal cycler (GeneAmp 2720, Applied Biosystems, Foster City, CA). Every 96-well plate also included an *E. coli* O157:H7 isolate known to be positive for all five of the targeted genes to serve as a positive control and a well containing only Go Taq Green, nuclease free water, and primers to serve as a negative control. The PCR products were separated by electrophoresis in 2 % agarose gels and visualized with UV illumination following staining with ethidium bromide. Isolates with a positive PCR reaction for *eaeA*, $fliC_{h7}$, *hly*A, *rfb*, *stx*-I and *stx*-II were designated as *E. coli* O157:H7. All *E. coli* O157:H7 positive isolates were stored in 15% glycerol at –80 °C for further characterization.

Pulsed Field Gel Electrophoresis (PFGE)

One isolate from each *E. coli* O157:H7-positive sample was characterized by pulsed field gel electrophoresis (**PFGE**). The PFGE typing was performed using the standardized Centers for Disease Control and Prevention (**CDC**) PulseNet protocol (Ribot et al., 2006). Briefly, isolates were grown on tryptic soy agar (TSA; Becton) plates and incubated at 37 °C for 18 h. Bacterial cultures were imbedded in 1 % agarose (SeaKem Gold Agarose, Cambrex Bio Science Rockland, Inc., Rockland, ME), lysed, washed, and digested with *Xba*I overnight at 37 °C. Restricted agarose plugs were then placed into 1 % agarose gels and electrophoresed on a CHEF Mapper XA (BioRad Laboratories, Hercules, CA) for 21 h with switch times of 2.16 s to 54.17s. *Xba*I-digested *Salmonella* ser. Braenderup (H9812) DNA was used as a reference size standard (Hunter et al., 2005). Agarose gels were stained in ethidium bromide and resultant images were captured with a FOTO/Analyst Investigator System (FOTODYNE, Inc., Hartland, WI). PFGE patterns were analyzed and compared using the Applied Maths Bionumerics v3.5 software package (Applied Maths, Saint-Matins-Latem, Belgium). Similarity clustering analyses were performed with Bionumerics software using the unweighted pairs group matching algorithm and the Dice correlation coefficient (Hunter et al., 2005).

Statistical Analysis

The trial will be conducted as a 2 x 2 factorial, completely randomized block design (RCBD) with repeated measures, consisting of four treatment groups with 63 animals per treatment, divided into 9 hd pens (n = 7 pens / treatment). Pens of cattle were treated as the experimental unit and individual cattle were treated as the sampling

unit. Data analysis was performed using GLIMMIX procedures in SAS (Statistical Analysis Software, v9.1.3, Cary, NC). The final model for prevalence of *E. coli* O157:H7 included the main effects of YC, MON, and sampling d as well as treatment x d interactions. The model also included a random statement to account for BW block and a repeated measure statement as all pens were sampled on multiple days. The three-way interaction of YC x MON x sampling day was included as the data would not converge. Differences were detected using the PDIFF statement in the model. A chi-square analysis using PROC FREQ in SAS was used to evaluate the association between fecal presence of *E. coli* O157:H7 and treatment. Frequency was determined by assigning either a "1" or "0" to those samples that did or did not, respectively, prevail with *E. coli* O157:H7. Individual comparisons for frequency differences within each day were analyzed using Fischer's exact test in SAS. The level of significance was set at $P < 0.05$.

RESULTS

One steer from the MON treatment was treated and then removed on d 110 for respiratory reasons that resulted in death; this was the only incidence of morbidity or mortality in the study. There was no effect ($P > 0.05$) of treatment on daily feed intakes which were 10.4, 10.2, 10.4, and 9.7 kg dry matter /d for CON, YC, MON, and Y x M treatment groups, respectively (data not shown). Initial cumulative prevalence of *E. coli* O157:H7 in environmental samples before cattle were either exposed to treatment pens or diets (d 0) are shown, by treatment group, in Figure 1. Six environmental isolates came from feed bunks (7.0 % prevalence) and pen floors (14.3 % prevalence); *E. coli* O157:H7 was presumptively not present in water samples. The proportion of cattle detected

shedding *E. coli* O157:H7 at each collection d is shown in Figure 2. Initial cumulative prevalence of fecal samples from pens of cattle before the initiation of dietary treatments began (d 0) was 71.4 % (20 / 28). Overall, 19.6 % (33 / 168) of fecal samples collected were presumptively positive for *E. coli* O157:H7. Cumulatively, 75.0 % (21) of the 28 pens had at least one positive sample over the course of the study. While there was no difference ($P > 0.05$) in fecal prevalence of *E. coli* O157:H7 due to dietary treatment, average pen prevalence of cattle shedding *E. coli* O157:H7 decreased significantly over time ($P < 0.01$). Additionally, while not significant, YC-fed cattle had 0 % prevalence of the pathogen by d 28, which sustained through d 84. Estimated probability of encountering the pathogen decreased ($P < 0.01$; Figure 3) over time in pooled pen samples.

Eight unique *Xba*I-PFGE subtype patterns were identified from 47 isolates confirmed to be *E. coli* O157:H7 from 39 positive environmental and fecal samples. A predominant PFGE subtype, subtype A, accounted for 22 % of all isolates characterized (Table 2). Additionally, PFGE subtypes B, C and F were characterized to be less than 3 bands different from the predominant subtype and were therefore considered to be closely related (Tenover et al., 1995). The remaining 3 subtypes differed from subtype A by ≥ 4 bands and were therefore not considered to be closely related. Cumulatively, the related subtypes (A, B, C and F) accounted for 78.7 % of *E.coli* O157:H7 isolates analyzed, were present in the feces of pens cattle from all treatment groups, and were present principally on d 0, with only subtype A persisting through d 28 and reappearing on 110 (Table 3). An event on or before collection d 110 introduced new subtypes D and H in the feces of cattle (Table 3). Dendogram banding patterns from PFGE characterization of *E. coli*

O157:H7 isolates indicating subtype, dietary treatment, collection day, and origination (environmental versus fecal) of samples are shown in Figure 4.

DISCUSSION

The current study was conducted as a companion to a larger project designed to test the effects of YC and MON on growth performance and carcass merit of heavy-weight finishing beef steers (Swyers et al., 2009). In the performance study, DDGS, a co-product from the ethanol industry, was included in the finishing rations not only due to price competitiveness, but also because DDGS are higher in fat, fiber and protein concentration and lower in starch on a DM-basis than corn (NRC, 2000). Additionally, in an effort to target current industry feeding practices, the inclusion level of DDGS (19.7 %, dry-matter basis) used in the finishing diets was based off of figures provided by a survey conducted by Vasconcelos and Galyean (2007). The survey indicated that 82.76% of beef nutritionists are currently formulating rations to include 5 – 50% ethanol by-products (DM basis) in finishing rations (average = 16.5%; mode = 20%).

Previous reports indicate that there is a positive association between feeding cattle fermented co-products (distiller's or brewer's grains) and the fecal shedding of *E. coli* O157. Synge et al. (2003) reported that supplementing the diet of beef cows with distiller's grains influenced higher fecal shedding rates of the pathogen than cows not given the co-product. In agreement, Dewell et al. (2005) reported that cattle fed brewer's grains were six times more likely to have *E. coli* O157-positive fecal samples than cattle not fed the co-product. Most recently, Jacob et al. (2008a, 2008b, 2008c) indicated that experimentally inoculated calves fed DDGS had higher hindgut (cecum, colon, and

rectum) persistence and fecal prevalence of *E. coli* O157:H7 than calves fed no DDGS. Similarly, in naturally-infected cattle, Jacob suggested that an observed twofold increase in fecal prevalence of the pathogen implies that the organism seems more likely to persist and colonize the hindgut more readily when DDGS are fed compared to when they are not. These reports indicate that supplementing the diet of beef cattle with DDGS may provide nutritive constituents that may directly or indirectly stimulate the growth of *E. coli* O157:H7. In contrast, Jacob et al. (2009) most recently reported no effect of diet on prevalence of the organism when 25% distiller's grains were fed to finishing cattle. The authors of the 2009 study concluded that a combination of low prevalence of the pathogen and variability of nutrient content in the distiller's grain may have attributed to the disparity seen between the 2009 trial and their earlier experiments.

Overall, there was no effect of treatment on fecal shedding of *E. coli* O157:H7 in pooled pen samples of naturally-infected heavy-weight finishing beef steers. The lack of effect due to MON was expected and is in agreement with previous reports indicating that ionophores either have no effect or exacerbate the prevalence of *E. coli* 0157:H7 shed in the feces of ruminants (Garber et al., 1995; Dargatz et al., 1997; Herriott et al., 1998; Edrington et al.; 2003). On the other hand, we expected to find that the YC would have an effect. While there is a lack of peer-reviewed work implementing YC, previous work using other direct-fed microbials (DFM), such as *Lactobacillus acidophilus*, has been effective in the preharvest control and intervention of *E. coli* O157 (Lema et al., 2001; Brashears et al., 2003; Elam et al., 2003; LeJeune and Wetzel, 2007; Tabe et al., 2008). Medellin-Pena et al. (2007) demonstrated that *L. acidophilus* secretes a molecule that inhibits or directly interacts with the virulence-related gene expression of *E. coli*

O157:H7. It is acknowledged that the mode of action of YC and *L. acidophilus* is likely different; therefore, the goal in offering prebiotics, such as the YC used in the current study, is to provide the native microflora (such as native *L. acidophilus*) with nutritive constituents that would aid in the prevention of the establishment of the pathogen (Callaway et al., 2003). With that said, we did not expect synergy with the parallel application of the prebiotic (YC) and the antibiotic (MON) (Steer et al., 2000). We expected that if the YC treatment had an effect, there would be contradictory results when YC was fed along with MON, and it would therefore be an incompatible strategy to offer both feed additives as an intervention of *E. coli* O157:H7. However, this was not observed in the current study so such conclusions cannot be drawn.

Regardless of treatment, we thought it possible that the low prevalence in the current study could be attributed to antibiotic residue inherent in the distiller's co-product which may have antagonized the growth of *E. coli* O157:H7. Jacob et al. (2008) previously discussed that in the production of ethanol, antimicrobials, such as penicillin G, streptomycin, tetracycline, monensin, and virginiamycin are employed to suppress bacterial growth (Day et al., 1954; Aquarone, 1960; Narendranath et al., 2000). While there is no evidence to suggest that the DDGS used in the current study contained antibioitic residue sufficient to destroy the pathogens, this may explain the lack of prevalence observed post d 0. However, there could be other properties inherent in the co-product, such as a dense population of lactobacilli spp. (Pederson et al., 2004), that was effective at preventing persistence of *E. coli* O157:H7. Because the study design lacked a treatment group receiving no DDGS, comparisons with or without DDGS in the diet cannot be made and therefore a conclusion cannot be clearly drawn.

While the cattle enrolled in the current study were asymptomatic, showing no outward signs indicating infection of *E. coli* O157:H7, the majority of the animals (71.4 %) entered the study with a natural infection of the pathogen. Therefore, due to the high incidence of natural infection that the cattle had upon entering the study along with the inclusion of DDGS in the finishing diets, we thought it possible that fecal prevalence of *E. coli* O157:H7 would be high and increase over the course of the study in CON-fed cattle. However, in disagreement with our hypothesis the prevalence of *E. coli* O157:H7 decreased over the course of the study with no difference detected due to dietary treatment. As afore mentioned, because there was not a treatment group receiving no DDGS, and therefore comparisons to a group of cattle not receiving DDGS could not be made, the DDGS appeared to not influence high shedding rates of the pathogen.

Aside from d 0, the prevalence of *E. coli* O157:H7 was quite low throughout the trial, displaying only a subtle spike in all treatment groups on d 110. Previously, Khaitsa et al. (2003) described three distinct prevalence phases of fecal shedding of *E. coli* O157:H7 in finishing beef steers when an epidemic curve-like pattern of the infection was observed. The 3 defined phases were described as pre-epidemic, epidemic, and post-epidemic and were characterized by a pattern of low initial prevalence, followed by dramatically high incidence, preceded by a second low prevalence, respectively. The authors of that study indicated that the pattern indicated time-dependent risk factors that could likely attribute to fecal shedding of *E. coli* O157:H7. This could explain the initial peak observed on d 0 of the current study, which was followed by a dramatic decrease of *E. coli* O157:H7 in the feces on d 28, and then a subtle spike on d 110. Besser et al. (1997) reported that the typical duration of fecal shedding of the pathogen to be

approximately 1 month, which was approximately the duration that spanned between when the cattle were purchased and received before being selected and in-processed for the current study. It seems likely that the cattle entered the trial during an epidemic, which was followed by a post-epidemic phase once initiation of treatments began. This theory coincides with the incidence of the predominant, related subtypes A and C that were observed in both the feces of cattle and environmental samples on d 0. It could be elucidated that these subtypes may have been unique to the facilities at SECRC and that the cattle were naturally-infected with these subtypes upon entering the feed yard (but not their treatment pens) the month prior. This is in agreement with previous reports indicating that the finishing unit rather than introduction of new cattle is the source of *E. coli* O157, particularly when there is persistence of the pathogen on environmental surfaces (Lahti et al., 2003).

It is also worth noting that the pattern of prevalence of *E. coli* O157:H7 observed in the feces of cattle coincided with changes in the weather pattern. The 125 d trial began in June and ended in October. During the trial, the average temperature was 19.4, 23.9, 27.8, 22.2, and 15.6 °C for June, July, August, September, and October, respectively (NOAA, 2008). These changes in temperature may be relevant, particularly in explaining an event that occurred on or before d 110 that caused not only a subtle spike in prevalence, but may have also attributed to the introduction of genetically diverse subtypes D and H. The D and H subtypes were not only > 7 bands different from the predominant subtype A, but were also distinctly different from each other. These 2 unique subtypes were present in CON, YC, and MON-fed cattle with the only similarity between the pens of cattle expressing these strains, aside from weather, was basal

feedstuffs. Therefore, it seems likely that the D and H subtypes could have been introduced from the diet or during handling or delivery of feed from the mill to the bunk and could account for the incidence observed on d 110. Infection of *E. coli* O157:H7 from contaminated food or water sources has been previously reported (Callaway et al., 2002; Dodd et al., 2003).

Limitations encountered in the current study included small number of sample collection days, low statistical power to detect differences, and low prevalence of *E. coli* O157:H7 in pooled samples may have reduced our ability to detect differences due to dietary treatment. The low detection of pooled pen samples (past d 0) is not surprising, because the sensitivity in detection methods decrease when *E. coli* O157-positive fecal samples are mixed with *E. coli* O157-negative samples (Sanderson et al., 2005). Lastly, it is worth mentioning that we noticed a high degree of a morphologically-similar strain (opaque, non-sorbitol fermenting spherical colonies with a defined center) throughout the study that was missing the H7 gene, and therefore was considered to lack the pathogenicity of *E. coli* O127:H7 and was not further evaluated in this study.

CONCLUSION

Under the conditions of this experiment, the feeding of YC and MON had few significant effects on fecal prevalence of *E. coli* O157:H7. However, overall average prevalence of *E. coli* O157:H7 was low (< 10 %) across all treatment groups, which might have affected our ability to find statistical differences. While the results of our research agreed with most reports concerning MON, there was no evidence to support that DDGS stimulated proliferation and shedding of the pathogen in the current study.

There was indication that supplementing the diets of finishing cattle with YC could decrease, but not eliminate, fecal shedding of *E. coli* O157:H7 upon initial application of the feed additive. More research to evaluate variable dosage levels of YC, with differing ration components (for example, with and without co-products), and the introduction of the feed additive into the diet at different times before slaughter is needed.

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TABLES AND FIGURES

Table 1. Ingredient and nutrient composition of diets on a dry-matter basis[1]

	Transition[2]				Finishing[3]			
Ingredients or nutrients	CON	YC	MON	Y x M	CON	YC	MON	Y x M
Ingredients, %[4]								
Alfalfa Hay	9.3	9.3	9.3	9.3	5.5	5.5	5.5	5.5
Corn silage	12.6	13.2	12.6	13.2	9.0	9.0	9.0	9.0
Flaked Corn	57.8	56.9	57.7	56.9	63.5	63.5	63.5	63.5
DDGS[5]	18.1	18.3	18.1	18.3	19.7	19.7	19.7	19.7
Limestone	1.5	1.5	1.5	1.5	1.6	1.6	1.6	1.6
Corn Meal[6]	0.49	0.01	0.48	-	0.31	0.01	0.31	-
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Oil	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Trace mineral premix[7]	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Vitamin E	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016
Vitamin A	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
Yeast Culture[8]	-	0.526	-	0.526	-	0.311	-	0.311
Monesin[9]	-	-	0.015	0.015	-	-	0.016	0.016
Nutrients, %[10]								
Dry Matter	71.28	71.49	73.38	73.36	74.75	74.76	75.52	74.85
Crude Protein	12.75	13.18	13.28	12.78	14.09	13.90	13.96	13.77
Non-protein Nitrogen	0.40	0.31	0.26	0.29	0.61	0.40	0.33	0.35
Neutral detergent fiber	17.98	18.08	17.50	18.16	16.78	16.55	17.15	17.37
Ether extract	5.28	5.35	5.16	5.05	5.70	5.41	5.66	5.71
Calcium	0.72	0.66	0.59	0.65	0.82	0.80	0.83	0.73
Phosphorus	0.39	0.40	0.40	0.38	0.39	0.39	0.39	0.38
Sulfur	0.26	0.30	0.27	0.28	0.24	0.26	0.26	0.27

[1]Diets were formulated to meet or exceed nutrition requirements of finishing beef cattle (NRC, 2000). The diets consisted of a basal control (**CON**) diet, supplemented with or without yeast culture (**YC**; Diamond V® "XP", Diamond V Mills, Inc., Cedar Rapids, IA) and with or without monensin (**MON**; Rumensin®, Elanco, Division of Eli Lilly and Company, Greenfield, IN), and the two-way interaction of YC x MON (**Y x M**).

[2]Transition ration fed for d 0 - 8 of trial.

[3]Finishing ration fed for d 9 - 125 of trial.

[4]Ingredients are partitioned as percentage of total mixed ration (**TMR**) on a dry-matter basis.

[5]DDGS = dried distiller's grains.

[6]Both the YC and MON were offered in the total mixed ration place of an equal amount of cornmeal on a dry-matter basis.

[7]Formulated to provide the following on a DM basis: 0.70% Ca, 0.39% P, 0.78% K, 0.25% Mg, 0.25% S, 0.25% NaCl, 75 mg/kg Zn, 85 mg/kg Fe, 10 mg/kg Cu, 25 mg/kg Mn, 0.20 mg/kg Co, 0.25 mg/kg I, and 0.12 mg/kg Se.

[8]The yeast culture was offered at manufacturer's recommended inclusion rate of 56 g animal^{-1}·d^{-1} during transition phase and decreased to 28 g animal^{-1}·d^{-1} during finishing phase (2.8 g / kg of DMI).

[9]The ionophore was offered at the inclusion rate of 11 mg / kg of DMI during transition phase and increased to 33 mg / kg of DMI during finishing phase.

[10]Values reported for nutritional composition of diets are based off of nutrient analysis from TMR samples taken every 7 d throughout the study. Values reported are a percentage of dry matter.

Table 2. Description of pulse-field gel electrophoresis (**PFGE**) characterization of *E. coli* O157:H7 isolates from environmental and fecal samples across all sample collection days.

PFGE Type	Band Difference from Type A	No. of Isolates	No. of Pens[1]
A	Dominant Subtype	22	8
B	2	1	1
C	3	12	9
D	> 7	4	2
E	4	1	1
F	2	2	1
G	>7	1	1
H	>7	4	1
Total	-	47	-

[1]Indicates the number of combined fecal and environmental samples throughout the study that were positive isolates of *E. coli* O157:H7. Environmental samples were collected on d 0 (pretreatment) from each treatment pen floor, feed bunk, and automatic water trough. Fecal samples were collected from every animal by rectal palpation on d 0, 28, 56, 84, 110, and 125.

Table 3. Distribution of pulse-field gel electrophoresis (**PFGE**) subtypes for each dietary treatment during each collection day[1,2,3]

		Treatment			
Collection Day	Environment	CON	YC	MON	Y x M
0	A, C	A, C, G	A, C, E	A, B, C, F	A
28	-	A	ND	A	ND
56	-	NA	ND	NA	ND
84	-	ND	ND	ND	ND
110	-	D	D	H	A
125	-	NA	NA	ND	NA

[1]Diets were formulated to meet or exceed nutrition requirements of finishing beef cattle (NRC, 2000). The diets consisted of a basal control (**CON**) diet, supplemented with or without yeast culture (**YC**; Diamond V® "XP", Diamond V Mills, Inc., Cedar Rapids, IA) and with or without monensin (**MON**; Rumensin®, Elanco, Division of Eli Lilly and Company, Greenfield, IN), and the two-way interaction of YC x MON (**Y x M**).

[2]Environmental samples were collected only on d 0 (pretreatment) from each treatment pen floor, feed bunk, and automatic water trough, whereas fecal samples were collected from every animal by rectal palpation on d 0, 28, 56, 84, 110, and 125.

[3]ND = *E. coli* O157:H7 was not detected; therefore PFGE analysis was not conducted.

[4]NA = *E. coli* O157:H7-positive isolates were detected but not analyzed using PFGE.

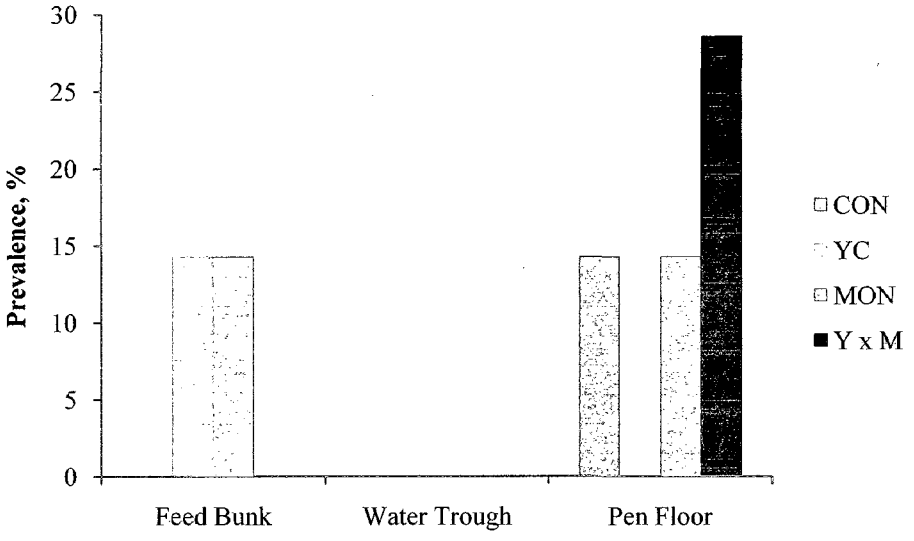

Figure 1. Prevalence of *E. coli* O157:H7 in environmental samples (feed bunk, water trough, and pen floor) taken on d 0 of trial before cattle were introduced to pens. On d 0 of the trial, before cattle were introduced to treatment pens, environmental samples were aseptically collected from each feed bunk, water trough, and pen floor from each pen that the animals were randomly assigned to be housed in for the duration of the study. *E. coli* O157:H7 was not detected in any samples taken from water troughs.

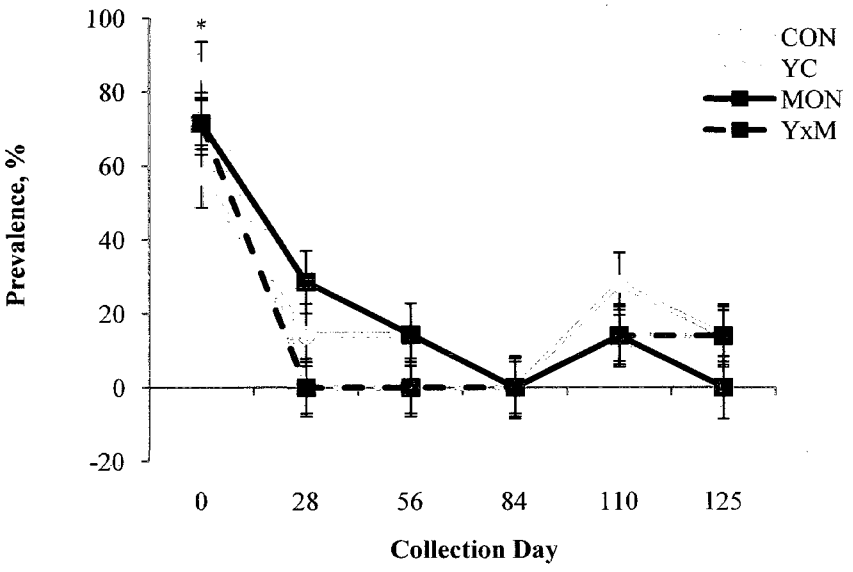

Figure 2. Prevalence and standard deviation of *E. coli* O157:H7 from d 0 (pretreatment) through 125 in fecal samples of pens of finishing beef steers (n = 7). Pens of steers were fed diets supplemented with or without yeast culture (YC) or monensin (MON) and the interaction thereof (Y x M). Both YC and MON were offered in the total mixed ration in place of an equal amount of cornmeal (DM basis; target intake = 2.8 g and 33 mg / kg of DMI, respectively). There was no difference due to treatment on fecal prevalence ($P > 0.05$), however there was a difference * due to day ($P < 0.01$). Error bars represent the standard deviation calculated for each dietary treatment for each collection day.

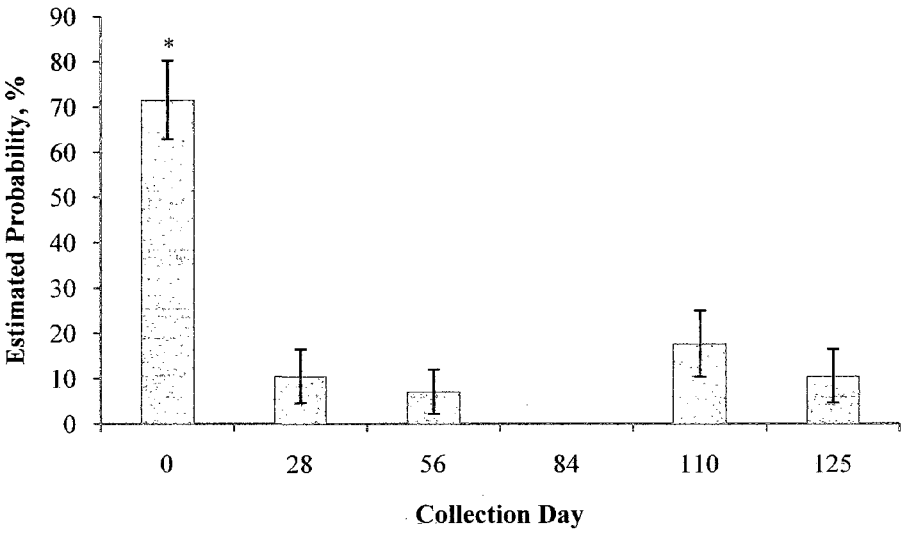

Figure 3. Estimated probability of encountering *E. coli* O157:H7 in the feces of cattle on each collection day across all treatments. Error bars represent the standard error calculated for each estimated probability. * Indicates difference detected in probability of encountering *E. coli* O157:H7 in the feces due to collection day ($P < 0.01$).

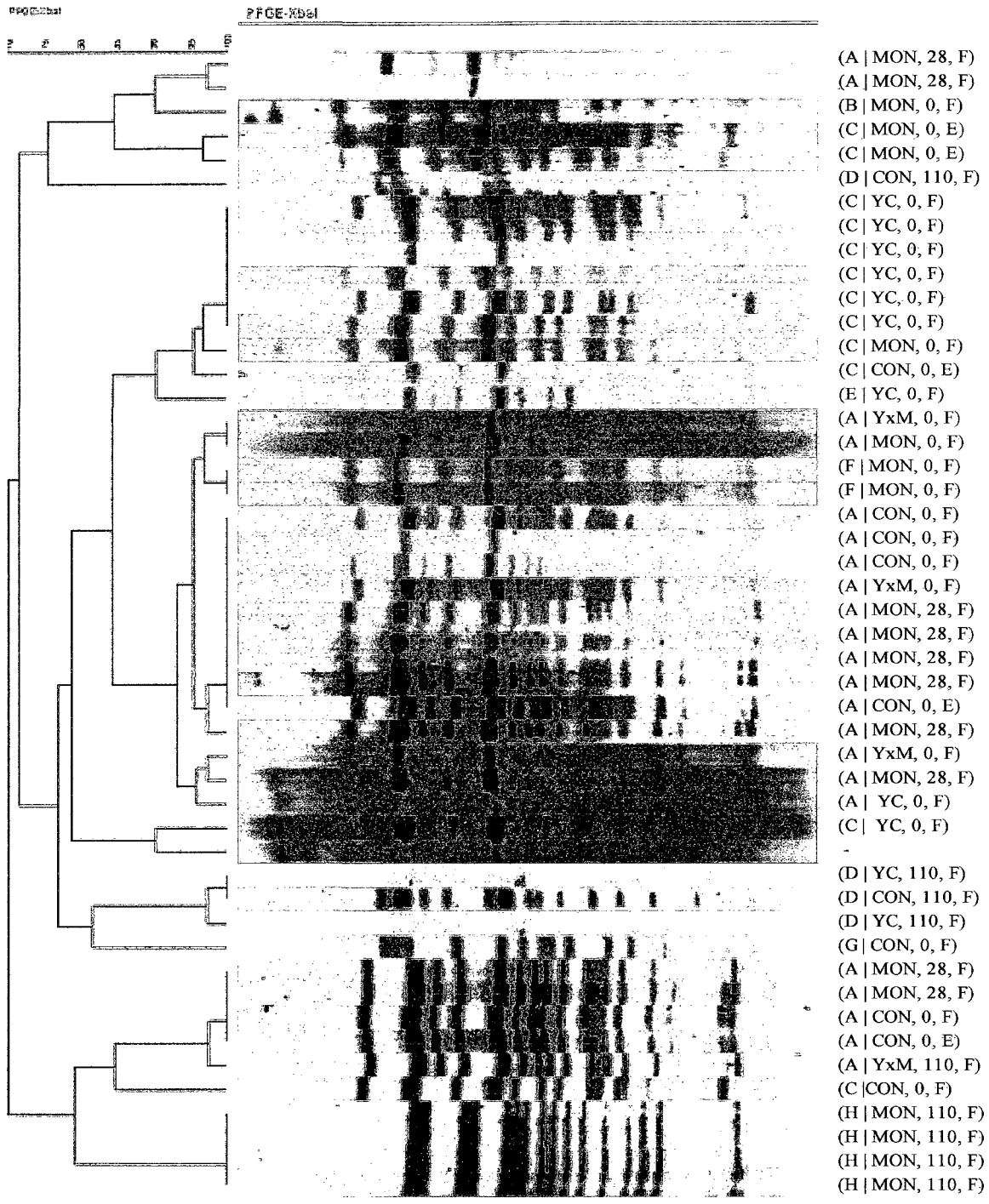

Figure 4. Dendogram banding patterns from pulse-field gel electrophoresis (**PFGE**) characterization of *E. coli* O157:H7 isolates from environmental and fecal samples across all sample collection days. The letters located to the right of each unique banding pattern

correspond to PFGE subtypes. Also indicated in parenthesis are the dietary treatment, collection day, and sample type that each isolate represents. Dietary treatments consisted of a basal control (**CON**) diet, supplemented with or without yeast culture (**YC**; Diamond V® "XP", Diamond V Mills, Inc., Cedar Rapids, IA) and with or without monensin (**MON**; Rumensin®, Elanco, Division of Eli Lilly and Company, Greenfield, IN), and the two-way interaction of YC x MON (**Y x M**). The sample types are either environmental (**E**) samples which were collected only on d 0 (pretreatment) from each treatment pen floor, feed bunk, and automatic water trough, or fecal (**F**) samples collected from pens of steers by rectal palpation on each collection day.