EVALUATION OF NATURAL FEED SUPPLEMENTS IN RUMINANT ANIMALS: THE EFFECTS ON FEEDLOT PERFOMANCE, CARCASS TRAITS, AND THE FECAL EXCRETION OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* SPP.

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ABSTRACT

Two studies were conducted to determine the effects of natural feed supplements, when included in the diet of feedlot animals. In the first study, ninety-six Dorset x Hampshire lambs (initial weight range 22.7 to 34.0 kg) were used in a complete block experiment with a 2 x 2 factorial arrangement of treatments to determine the effects of Amaferm® (**AMF**) supplementation in diets containing either a high proportion of starch (high concentrate; **CON**) or high proportion of fiber (high forage; **FOR**) on growth performance and carcass characteristics. Lambs were allotted to 24 pens (four lambs per pen) that were blocked by sex and weight. Lambs were fed until the average live weight of each pen reached a target weight (55.4 kg for wethers and 50.0 kg for ewes), at which time the entire pen of lambs was harvested. Lambs that received the CON diet consumed less ($P < 0.05$) average dry matter intake (DMI) (1.42 vs. 1.93 kg) and produced carcasses that had greater ($P < 0.05$) backfat (0.80 vs. 0.65 cm), body wall (2.36 vs. 1.95 cm), and ribeye area (15.13 vs. 13.31 cm^2). Additionally, the CON diet resulted in carcasses that received higher ($P < 0.05$) leg (12.23 vs. 11.37) and conformation (11.96 vs. 11.37) scores, as well as higher ($P < 0.05$) quality grades (12.46 vs. 11.67). Supplementation of AMF had no effect ($P < 0.05$) on carcass characteristics. The results of feedlot performance indicated that an interaction occurred between energy source and feed supplement ($P < 0.05$). For lambs that received the CON diet, AMF

supplementation resulted in improved ($P < 0.05$) feed efficiency (0.257 vs. 0.245 kg gain/kg feed) and greater ($P < 0.05$) average daily gain (ADG) (0.37 vs. 0.35 kg/d). However, for lambs that received the FOR diet, AMF supplementation resulted in less ($P < 0.05$) efficient feedlot performance. Results indicate that at the levels fed, AMF may improve growth performance for lambs finished on high concentrate diets, but not high forage diets.

In the second study, one hundred sixty-eight crossbred beef steers, initially weighing 250 to 340 kg, were used in a trial with a 3 x 2 factorial arrangement of treatments. The study was designed to examine the effects of natural feed supplements (Levucell SB (**LEV**) and Amaferm ® (**AMF**)) with two dietary energy sources (dry whole shelled corn (**DWSC**), or high moisture corn (**HMC**)) on growth performance, carcass characteristics and the ability of these products to reduce fecal excretion of *E. coli* 0157:H7 and *Salmonella* spp. of fed cattle.

Cattle receiving HMC showed an improvement ($P < 0.05$) in feed efficiency compared to those cattle being fed DWSC (0.212 vs. 0.202 kg gain/kg feed) regardless of feed supplement. Addition of LEV to the high concentrate corn-based diets fed in this study did not have an impact ($P > 0.05$) on growth performance of feedlot steers. However, addition of AMF to a diet composed of DWSC resulted in an improvement ($P < 0.05$) in feed efficiency (0.208 vs. 0.194 kg gain/kg feed). Neither of the feed additives, LEV or AMF, had an affect on carcass characteristics, nor did they reduce the incidence or apparent fecal shedding of *E. coli* O157:H7.

No animals tested positive for *Salmonella* throughout the feeding period. A spike in the fecal excretion of *E. coli* O157:H7 was seen approximately 6 weeks after the start

of the trial. Differences ($P < 0.05$) in the excretion of *E. coli* O157:H7 by feedlot cattle were observed between the two corn sources fed in this study. Cattle being fed HMC appeared to have more resilience against infection of *E. coli* O157:H7 as the percent of animals within a pen excreting *E. coli* O157:H7 for pens receiving HMC declined to levels near those seen prior to the peak; whereas, the average percent of cattle within a pen for those pens on the DWSC diet was greater for several weeks after the peak in fecal shedding occurred. Although statistical significance was not determined, it appeared as if steers fed HMC excreted *E. coli* O157:H7 less frequently than steers fed DWSC, which may imply that these animals were less susceptible to infection. This study provides evidence that cattle finished on a high concentrate diet that utilizes high-moisture corn may exhibit a lower level of excreting *E. coli* O157:H7 and may be more resilient in returning to low levels of fecal excretion after a challenge or stress initiates a spike in excretion compared to cattle finished on a high concentrate diet formulated with dry, whole shelled corn throughout the finishing period.

Dedicated to my family

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CHAPTER 1

INTRODUCTION

It is estimated that 76 million cases of food-borne illnesses occur in the United States each year as a result of contaminated food (Mead et al., 1999). The causes of these illnesses include bacteria, viruses, parasites, toxins, metals and prions (Mead et al., 1999). Two of the most notable pathogenic bacteria responsible for the occurrence of food-borne illnesses, receiving increased public attention, are *Escherichia coli* O157:H7 and *Salmonella* spp. *E. coli* O157:H7 is the most common enterohemorrhagic *E. coli* serotype found in human cases of food related illnesses in the U.S. and is thought to act through the production of one or more verotoxins, or Shiga toxins (Griffin and Tauxe, 1991; Jay et al., 2005). Exposure to livestock, particularly cattle, and consumption of contaminated foods of bovine origin have often been identified as risk factors for developing illness from this organism (Loneragan and Brashears, 2005). Ingestion of this pathogen can cause bloody diarrhea in humans, but can also lead to more serious infections such as hemorrhagic colitis and hemolytic uremic syndrome (HUS). HUS, which develops in about 8% of infected individuals, is characterized by the sudden onset of hemolytic anemia, with fragmentation of red blood cells, thrombocytopenia, and acute renal failure (Weir, 2000). Although the occurrence of *E. coli* O157:H7 infections

accounts for only 0.5% of total food-borne illnesses in the United States, an outbreak of this pathogen in 1993 that resulted in the death of three children has brought much attention to this pathogen and its increased frequency of occurrence over the past several years (Bell et al., 1994; Mead et al., 1999). The occurrence of *Salmonella* spp. as the source of food-borne illnesses is even greater than *E. coli* O157:H7 and is second only to *Campylobacter* in causing bacterial gastroenteritis in humans (Mead et al., 1999). Infections with *Salmonella* can be severe and account for the highest percentage of total food-related deaths at 30% (Mead et al., 1999).

Food-borne outbreaks due to *E. coli* O157:H7 and *Salmonella* have frequently been linked to the consumption of contaminated meat products and other foods of animal origin. An outbreak in 1994 associated with contaminated raw ground beef resulted in 107 confirmed cases of *Salmonella* infections (CDC, 1995). Additionally, contamination of frozen ground beef burgers with *E. coli* O157:H7 and contamination of hamburgers from a fast-food chain resulted in 15 and 10 cases of infection, respectively (CDC, 1996, 1997).

Previous studies have identified livestock to be transient carriers of pathogens such as *E. coli* O157:H7 and *Salmonella* spp. (although some cattle can be chronically infected with *Salmonella*) (Meyer-Broseta et al., 2001; Jay et al., 2005). These animals frequently excrete these pathogens in feces, which allows these microorganisms to be transmitted to the hide and oral cavity of other animals and continues the life cycle of infection (shedding). Elder et al. (2000) reported that fecal and hide prevalence of *E. coli* O157:H7 in live cattle was significantly correlated with the prevalence of subsequent post-harvest carcass contamination. Therefore, strategies that could be implemented on

the farm, or prior to harvest, that would eliminate or reduce these pathogens in the live animal could enhance food safety throughout the remainder of the processing system and supply chain. A variety of pre-harvest strategies have been evaluated on farms for their ability to reduce pathogenic microorganisms in the live animal. However, from a producer's standpoint, these interventions are just another unnecessary production cost, because they do not receive any benefits by decreasing pathogen numbers in their animals. Producers, on the other hand, are looking for ways to improve their efficiency of production so that they may capture the greatest amount of profit. Therefore, strategies need to be developed that both reduce the presence of pathogenic bacteria, while also improving animal performance and production efficiency.

Probiotics and prebiotics may be capable of reducing pathogenic microorganisms in live animals. A probiotic is defined as a live microbial feed supplement that benefits the host b y improving it's intestinal microbial balance (Fuller, 1989). A prebiotic, on the other hand, is a nondigestible nutritional compound that selectively stimulates the growth of the endogenous microflora within the digestive tract (Walker and Duffy, 1998). Both of these compounds have been shown by many researchers to selectively increase populations of beneficial microorganisms within the rumen (Newbold, 1995; Yoon, 1996; Harper et al., 1996; Beharka and Nagaraja, 1998; Krehbiel et al., 2003). Through the stimulation of beneficial microbes, these compounds may be capable of reducing populations of pathogenic microorganisms by out competing pathogens for nutrients and places of attachment within the gastrointestinal tract.

Amaferm® (Biozyme Inc., St. Joseph, MO) is feed supplement that has been identified as a prebiotic. Many researchers have reported an increase in the population of

fiber digesting and lactate utilizing bacteria, as well as an increase in the population of rumen fungi when Amaferm, which is a fermentation extract of the mold *Aspergillus oryzae*, was fed in ruminant diets (Gomez-Alarcon et al., 1990; Beharka, et al., 1991; Beharka and Nagaraja, 1993; Harper et al., 1996; Beharka and Nagaraja, 1998; Chang et al., 1999). Moreover, Gedek et al. (1999) reported the ability of the yeast *Saccharomyces cervisiae* ssp. *boulardii* to irreversibly bind *E. coli* O157:H7 and *Salmonella Typhimurium* DT104 to its outer membrane. Levucell SB (Lallemand Nutrition, France) is a probiotic feed supplement that contains the CNCM I-1079 strain of *Saccharomyces cervisiae*, ssp. *boulardii* and may have the ability to prevent adhesion of these pathogens to tissue surfaces and facilitate the removal of these microorganisms from the gastrointestinal tract.

In addition to reducing the prevalence of pathogens in ruminant animals, there is reason to believe that probiotics and prebiotics may have a positive impact on animal performance and feed efficiency by increasing digestibility of feedstuffs through increased microbial populations. An improvement in production efficiency would give producers an incentive to use these products in their livestock operations. However, although several studies have demonstrated the impact of Amaferm supplemention on digestibility of feedstuffs, few studies have examined the direct effects of probiotics and prebiotics on feedlot performance. Additionally, no studies have reported the effects of prebiotics and fungal probiotics on the fecal excretion of *E. coli* O157:H7 and *Salmonella* spp. by feedlot ruminant animals. This thesis is an investigation of the effects of natural feed supplements (probiotics and prebiotics) on growth performance, carcass traits, and the fecal excretion of *E. coli* O157:H7 and *Salmonella* in ruminant animals.

CHAPTER 2

REVIEW OF LITERATURE

POST-HARVEST INTERVENTIONS

The meat industry has worked extensively in combating food-borne pathogens by developing several methods to reduce contamination of carcasses and the products from these carcasses. Focus has primarily been on post-harvest reduction of pathogens or improving the safety of meat products after slaughter. Several treatments have been developed and tested to determine their effectiveness in decontaminating carcasses during the harvesting process. Previous reports have shown the ability of hot water (80 to 95°C) to reduce microbial levels on carcasses (Smith and Graham, 1978; Barkate et al., 1993; Dorsa et al., 1996; Dorsa et al., 1997; Castillo et al., 1998). Smith and Graham (1978) showed a 99% reduction in the numbers of *E. coli* and *Salmonella* on inoculated beef samples and sheep carcasses when treated with water at 80°C for 10 sec. However, hot water alone has been shown to be less effective than organic acids (lactic or acetic acid) at inhibiting growth during a 21 day storage period (Dorsa et al., 1997). Three percent lactic acid was the most effective treatment in inhibiting growth of *E. coli* O157:H7 after 21 days of refrigeration storage (Dorsa et al., 1997). Hardin et al. (1995) also found

lactic acid to be more effective than acetic acid in reducing *Salmonella* Typhimurium and *E. coli* O157:H7 from inoculated beef carcasses.

Several studies have used two or more methodologies in combination to determine if multiple interventions would cause more significant reductions than any one alone. Castillo et al. (1998) compared the effects of a high pressure water wash or trimming combined with hot water and warm lactic acid spray treatments and found that water wash or trim, followed by hot water and then lactic acid spray was most effective in reducing fecal or pathogenic contamination.

Other methodologies have employed steam vacuuming and steam pasteurization for decontamination of beef carcasses. Dorsa et al. (1996) tested the effects of a steam vacuuming system designed to deliver > 82.2°C water plus steam directly to the carcass surface, while physically removing contamination through a vacuum. The steam vacuum was successful in reducing bacterial numbers and produced even larger reductions of 3.1, 4.2, and 4.3 $\log_{10}$ CFU/cm^2 for aerobic plate count, coliforms, and *E. coli* populations, respectively, when combined with a double water wash following its application (Dorsa et al., 1996). Additionally, steam vacuuming applied to carcasses prior to evisceration was effective in removing microbial contamination on carcasses with and without visible fecal contamination (Kochevar et al., 1997).

Steam pasteurization of carcasses for 8 seconds, applied as the last step of the slaughter process, reduced aerobic plate counts by 1.35 and 1.11 $\log_{10}$ CFU/cm^2 on fed cattle and cow carcasses, respectively, in a commercial facility (Nutsch et al., 1997). In addition, 16.4% of the carcasses were positive for generic *E. coli* before pasteurization, compared to 0% of the carcasses after treatment. An important advantage of steam

pasteurization is that it does not require the use of chemicals and is not hazardous to plant personnel or equipment if used properly (Nutsch et al., 1997).

Post-harvest intervention strategies, together with hazard analysis and critical control point methods have been very effective in reducing the presence of food-borne pathogens within the meat industry. All major slaughter facilities are currently required to implement HACCP policies and most operations use one or more post-harvest treatments to reduce carcass contamination. However, food-borne illnesses still occur and meat products continue to be the source in several cases.

PREVALENCE OF PATHOGENS IN CATTLE AND SHEEP

Location within the gastrointestinal tract

A probable reason for explaining why such high numbers of food-borne illnesses are associated with contaminated meat products may be due to the fact that domestic farm animals, such as cattle and sheep, transiently carry these pathogenic microorganisms within their gastrointestinal tracts (Meyer-Broseta et al., 2001; Jay et al., 2005). *E. coli* O157:H7 and *Salmonella* spp. have been located at various locations within the animal, but have been shown to primarily colonize in the colon and lymph nodes, respectively (Grauke et al., 2002; Jay et al., 2005). In fact, *E. coli* O157:H7 seems to colonize the lower gastrointestinal tract, or hindgut, of ruminant animals as it is rarely cultured from digesta or tissue in areas of the upper gastrointestinal tract, including the rumen (Buchko et al., 2000a; Grauke et al., 2002). Although this microorganism is pathogenic to humans, cattle and sheep are asymptomatic carriers of *E. coli* O157:H7, as they appear healthy and free of disease, even when actively excreting the organism in feces (Grauke

et al., 2002). *Salmonella* spp., on the other hand, can cause disease in animals and symptoms include diarrhea, fever, and chronic weight loss. However, similar to *E. coli* O157:H7, most infections are subclinical, in which cattle actively excrete *Salmonella* spp. in feces, but do not show clinical signs of infection.

Estimated prevalence in feedyards

Reports on the prevalence of *E. coli* O157:H7 and *Salmonella* in cattle and sheep have varied. In a study designed to estimate the prevalence of *E. coli* O157:H7 in feedlot cattle in the USA, Hancock et al. (1997) isolated *E. coli* O157 from 210 (1.8%) of 11,881 fresh fecal-pat samples from 100 feedlots in 13 states. Of 24,184 samples collected from fresh fecal pats on pen floors, drinking water, and feed rations in 4 feedyards in Southwest Kansas, only 0.19% of samples were positive for *E. coli* O157:H7 (Galland et al., 2001). However, other estimates have reported the prevalence of *E. coli* O157:H7 in fresh fecal pats collected throughout the cattle feeding period to be as high as 13.3% (LeJeune et al., 2004). The differences in estimated prevalence may be due to the differences in culture and sampling methods between each of the studies. In the study by LeJeune et al. immunomagnetic separation was performed to concentrate *E. coli* O157:H7 from enriched cultures, however, this procedure was not performed in the study by Hancock et al. or the study by Galland et. al.

Seasonal variation has also been found to directly impact the incidence of *E. coli* O157:H7-positive animals (Kudva et al., 1996; Barkocy-Gallagher et al., 2003). Thus, the number of animals found to be excreting a particular bacterium may depend on the time of year in which the animals are being tested. Kudva et al. (1996) found 31% of

sheep sampled in June to be positive for *E. coli* O157:H7, however, when those same animals were sampled in August and November only 5.7 and 0% tested positive, respectively. This seasonal variation may partially account for the variation in estimates of the prevalence of *E. coli* O157:H7 and *Salmonella* that have previously been reported.

The excretion of *E. coli* O157:H7 in cattle feces has been shown to be sporadic, meaning that it occurs at irregular intervals throughout the finishing period. Several studies, though, indicate that the prevalence of *E. coli* O157:H7 is higher early in the feeding period. Cattle that are initially placed into a feedlot encounter many stresses, including adaptation to a different environment and diet, which can make them susceptible to infection with pathogenic microorganisms. Dargatz et al. (1997) observed that if a pen of cattle within a feedlot had been on feed less than 20 days there was an increased likelihood of that pen being positive for *E. coli* O157. These findings were supported by those of LeJeune et al. (2004) in which it was found that overall prevalence of *E. coli* O157:H7-positive fecal samples peaked 2-weeks after entering the feedlot. When comparing the amount of time in which pens within a feedlot have been on feed it was found that the prevalence of *E. coli* O157:H7 was threefold higher in pens of cattle that had been on feed the shortest compared to those pens of cattle that had been on feed the longest (Hancock et al., 1997).

In addition to differences in the duration of time in which animals excrete food-borne pathogens, the number of times in which an animal becomes infected also varies. Ruminant animals have been known to excrete *E. coli* O157:H7 in feces for a period of time, followed by a period in which the microorganism is not detectable in feces, followed by periods in which the pathogen reappears in feces. However, prior exposure

to *E. coli* O157:H7 does not seem to have a significant impact on the gastrointestinal tract location or duration of excretion of the bacteria upon further infections, although this has not been definitively identified (Grauke et al., 2002; Khaitsa et al., 2003). Kudva et al. (1997) also found that previous colonization of *E .coli* O157:H7 within the gastrointestinal tract of ruminants does not prevent recolonization of the bacterium. So although these microorganisms are not pathogenic to ruminant livestock, these animals possess neither long-term, nor short-term defense against attachment and colonization of these microorganisms within their gastrointestinal tract (Khaitsa et al., 2003).

Estimated prevalence prior to or during harvest

As the pathogen load entering abattoirs on harvest-ready cattle and sheep increases, the possibility of carcass contamination and the likelihood for post-harvest intervention failures increases as well. For this reason, several studies have looked at the prevalence of *E. coli* O157:H7 and *Salmonella* in ruminants just prior to or during harvest. In a study conducted at meat processing plants in the Midwestern United States, 28% and 11% of feces and hides, respectively, were found to be positive for *E. coli* O157 during processing (Elder et al., 2000). Similar findings on the presence of *Salmonella* within the oral cavity and feces of cattle have also been reported. In a study in which 100 cattle were sampled prior to and during processing, *Salmonella* was isolated from 26% of samples, including 68% of hides, 29% of oral cavities, and 16% of feces (Fegan et al., 2005). Others have reported lower prevalence (15.4%) of *Salmonella* on beef hides immediately prior to their removal from the carcass (Bacon et al., 2002).

Hides have been reported to be the primary source for carcass contamination. Presence of *Salmonella* and *E. coli* O157:H7 in feces and in the environment serves as a source for contamination of hides and transmission of these pathogens to other animals within a group. In fact, prevalence of *Salmonella* and *E. coli* O157:H7 has been found to be greater on hides than in feces, possibly because of the ability of multiple cattle to be exposed to contaminated feces of just one animal (Fegan et al., 2005). Fegan et al. (2005) found no correlation between *Salmonella* in the rumen and feces and contamination of hides. When hide prevalence was high, isolation of *Salmonella* from feces and rumen content has been found to be low. These findings are supported by a study in which *E. coli* O157:H7 and *Salmonella* were isolated from 5.9% and 4.4% of fecal samples, respectively, but were isolated from 60.6% and 71.0% of hide samples, respectively (Barkocy-Gallagher et al., 2003). Therefore, although an animal may not be internally infected with *Salmonella* or *E. coli* O157:H7, it may contain the pathogen on its' hide if other animals in the pen are excreting the microorganism in their feces or if these animals rub against contaminated surfaces within the pen environment. Contradictory findings have been reported as Elder et al. (2000) found relatively low hide prevalence of *E. coli* O157 compared to feces. Younts-Dahl et al. (2004) found positive hide samples and fecal samples to be in agreement only 8% of the time, indicating that fecal status is not a good indicator of hide status and vice versa.

During processing, high prevalence of pathogenic microorganisms on hides increases the likelihood for subsequent carcass contamination (Loneragan and Brashears, 2005). Prevalence of *E. coli* O157 in feces and on hides of live animals is directly correlated with carcass contamination, especially initial carcass contamination (Elder et

al., 2000). In fact, one study showed that 90% of lots that had at least one positive fecal sample also had positive carcass samples prior to evisceration (Elder et al., 2000). A similar correlation exists between the presence of *Salmonella* on hides and oral cavities and further contamination of carcasses (Fegan et al., 2005). Thus, although post-harvest strategies that have been taken inside slaughter plants to reduce pathogenic bacteria have been very effective, opportunities to reduce these pathogens in the live animal may have even greater potential to improve the safety of meat products and reduce the incidence of human food-borne illnesses.

PRE-HARVEST INTERVENTIONS

Effect of diet on pathogen populations

In response to recent findings that the live animals is the primary source of subsequent carcass contamination, greater emphasis has recently been targeted toward the development of pre-harvest interventions, which focus on reducing pathogenic microbial populations in the live animal. Strategies that have been investigated range from supplementation of many types of feed additives in ruminant diets to the use of antibiotics and vaccines to simple on-farm management practices that could possibly reduce the risk of food-borne pathogens in livestock.

Although there has been no single management practice identified that is consistently associated with pathogen prevalence, the effect of diet on pathogen populations within feedlots has been extensively studied. Cattle are typically weaned at approximately 5 to 7 months of age. At weaning, cattle experience a shift from a diet consisting mainly of milk from their mothers and forages to a diet that is high in

carbohydrates. Following weaning and adaptation to a grain-based diet, cattle are placed into feedlots where they are fed high concentrate diets to maximize performance and animal production. These high concentrate diets, which consist mostly of grains, are fed until a targeted market weight is reached, at which time cattle are sent to harvest. Pre-harvest dietary management may play a role in the prevalence of food-borne pathogens in ruminant animals and thus, the type of diet cattle receive may impact the incidence of food-transmitted diseases.

The ability of a bacterium to act as a food-borne pathogen depends on its capacity to survive the low pH of the gastric stomach and to colonize the human intestinal tract (Gorden and Small, 1993). Grain contains high amounts of starch. Many forms of starch pass through the rumen to the intestines where little degradation takes place because cattle are deficient in the starch degrading enzyme, amylase (Diez-Gonzalez et al., 1998). Therefore, large amounts of starch reach the colon where it is fermented to produce volatile fatty acids (acetate, propionate, and butyrate) which decrease the pH in this area of the digestive tract. Diez-Gonzalez et al. (1998) reported that this decrease in pH allows *E. coli* to develop a resistance to the acidic environment of the colon, thus enabling the bacteria to proliferate in this region of the gastrointestinal tract. In support of these conclusions, Tkalcic et al. (2000) observed that ruminal fluid from steers fed a high-concentrate diet rapidly induced acid resistance in *E. coli* O157:H7 and concluded that this acid tolerance allows the bacterium to survive passage through the acidic abomasum to the colon, which could in turn influence fecal excretion in grain-fed cattle. In fact the colon has been shown to be the main site of *E. coli* O157:H7 proliferation (Grauke et al., 2002).

It has been found that grain feeding does in fact lower the ruminal and intestinal pH of cattle, which favors acid resistant bacteria such as *E .coli* O157:H7 and increases the presence of these potential pathogens within ruminant animals (Diez-Gonzalez et al., 1998). These acid resistant *E. coli* O157:H7 would then be able to survive the pH of the gastric stomach if ingested by humans through contaminated meat products, and thus act in causing food-borne disease.

In contrast to these results, Hodve et al. (1999) demonstrated that resistance of *E. coli* O157:H7 to acid shock was unaffected by diet, thus making the microorganism still susceptible to the pH of the human gastric stomach. Kudva et al. (1997) challenged that low fiber and high nutrient feeds, such as grains, do tend to increase the production of volatile fatty acids within the rumen which leads to a decrease in pH, however, this in turn leads to a less favorable environment for bacteria such as *E. coli* O157 when compared to high fiber low-nutrient feeds.

Several studies have looked at the incidence of *E. coli* O157:H7 and *Salmonella* between forage fed cattle and grain fed cattle and results have been conflicting. Cattle fed hay have been reported to excrete *E. coli* O157:H7 for a longer duration than grain fed cattle and one study found this duration to be almost twice as long (Kudva et al., 1997; Hodve et al., 1999). The concentration of *E. coli* O157:H7 in cattle feces was also found to be higher in cattle fed a forage diet when compared to cattle fed grain diets (Van Baale et al., 2004). On the contrary, the prevalence of *Salmonella* in lot-fed cattle was reported as double that of grass-fed cattle (Fegan et al., 2004). Additionally, the feeding of grain increased the population of *E. coli* in cattle when compared to those cattle that were fed medium-quality timothy hay (Diez-Gonzalez et al., 1998). When analyzing

grain-fed cattle, the incidence of *E. coli* O157:H7 may vary depending on what type of grain is being fed. The feeding of barley has been reported to increase the likelihood of a pen of cattle within a feedlot being positive for *E. coli* O157 compared to corn fed cattle (Dargatz et al., 1997; Buchko et al., 2000a; Berg et al., 2004). The feeding of barley not only increases the prevalence of *E. coli* O157:H7 but also increases the amount of the bacterium within feces compared to corn-fed cattle (Berg et al., 2004).

It has been suggested that manipulating the diet of the ruminant animal at the end of the finishing period, just prior to harvest may impact the prevalence and population of pathogenic microorganisms within the gastrointestinal tract. In a previous study using experimentally inoculated sheep there was an increase in the fecal excretion of *E. coli* O157:H7 when the diet was abruptly changed from a high-nutrient, low-fiber diet to a low-nutrient, high-fiber diet (Kudva et al., 1997). Additionally, decreased fecal excretion was observed when the reverse was performed (Kudva et al., 1997). However, other researchers have found limited effects on fecal excretion of *E. coli* O157:H7 when ruminants were subjected to an abrupt dietary change (Keen et al., 1999; Buchko et al., 2000b).

When analyzing the impact of diet manipulations on the fecal excretion of pathogenic microorganism, the economic impact must also be taken into account. Ruminants are fed high concentrate diets consisting of grains during the finishing period to maximize performance and feed efficiency. Cattle switched to 100% alfalfa hay 15 days prior to slaughter lost 0.11 kg/day while those cattle that remained on a grain diet gained approximately 0.45 kg/day (Keen et al., 1999). Therefore, abrupt changes in the diet may have undesirable impacts in terms of animal production efficiency.

Ionophore supplementation

The use of feed additives in animal production to further improve performance and efficiency is common practice within the livestock industry. In addition to these products increasing growth and performance, many may also impact the prevalence of food-borne pathogens through various mechanisms.

Ionophores are a class of antibiotics that were approved for use in ruminant diets in the mid-1970's by the Food and Drug Administration. Ionophores modify the movement of ions across biological membranes, primarily causing the entry of sodium (Na^+) ions into cells (Schelling, 1984). Although it is fully recognized that the use of ionophores increases animal performance, the mechanisms by which they do so continues to be under investigation. Proposed mechanisms through which ionophores improve performance include decreased methane production, decreased acetate to propionate ratio, modified feed intake, and decreased ammonia production from protein degradation (Schelling, 1984).

Because ionophores are not used in human medicine and have a distinctly different mode of action than therapeutic antibiotics, their use in livestock production is not likely to have a significant impact on antibiotic resistance in humans (Russell, 2003). However, there seems to be a temporal relationship between the initial use of ionophores in production livestock and the increase in food-borne infections due to *E. coli* O157:H7. In fact, Edrington et al. (2003a) reported the number of sheep excreting *E. coli* O157:H7 in feces was higher when sheep were fed the ionophores monensin or laidlomycin propionate compared to controls, although the difference was not significant. Similar results were found when monitoring fecal excretion of *Salmonella* Typhimurium

(Edrington et al., 2003a). On the contrary, Van Baale et al. (2004) found that cattle fed a forage diet had reduced excretion of *E. coli* O157:H7 when monensin was supplemented in the diet, however, no differences were observed among grain-fed cattle. Still, other studies have found monensin to have no effect on the growth of *E. coli* O157:H7 at low concentrations, but to significantly reduce the growth of *E. coli* O157:H7 at a concentration of 50 μg/mL (Bach et al., 2002b). Currently, ruminal concentrations of cattle fed monensin at recommended levels would be approximately 5-10 μg/mL. Thus, in order to be effective in reducing pathogens, monensin would need to be fed at levels 5 times that which it is currently fed, which may not be economically feasible or approved by the U.S. Food and Drug Administration.

Other feed additives

Several feed additives have been investigated for the sole purpose of their ability to reduce pathogenic bacteria populations in the live animal. Supplementation of sodium chlorate in the diet of ruminant animals has been proposed as a potential pre-harvest intervention to reduce food-borne pathogens in livestock populations. Most members of the *Enterobacteriaceae* family possess the enzyme nitrate reductase which converts nitrate to nitrite, allowing these microorganisms to respire anaerobically. However, this enzyme also reduces chlorate to the toxic metabolite chlorite, which is deadly to nitrate reductase-positive bacteria, such as *E. coli* O157:H7 and *Salmonella* (Anderson et al., 2000). In vitro studies using buffered ruminal fluid have shown concentrations of *E .coli* O157:H7 and *Salmonella* Typhimurium DT104 to be reduced upon addition of sodium chlorate (Anderson et al., 2000). Similar results have been found in vivo in which

sodium chlorate reduced *E. coli* O157:H7 populations in the rumen and feces of experimentally inoculated cattle (Callaway et al., 2002a). Anderson et al. (2000) suggest that sodium chlorate could be supplemented in the last meal prior to shipment of cattle to slaughter. In fact, *E. coli* O157:H7 populations were reduced in the rumen, cecum, and rectum of experimentally inoculated sheep supplemented with sodium chlorate ($NaClO_3$) for 24 hours prior to harvest (Callaway et al., 2003).

Although sodium chlorate supplementation reduced populations of *E. coli* O157:H7 in the rumen, cecum, and rectum, it has been shown that the primary site of proliferation of this pathogenic microorganism is the lower gastrointestinal tract. These findings that infection and proliferation of *E. coli* occurs primarily in the lower intestine sparked interest in the development of a sodium chlorate product that bypasses the rumen, thereby allowing the delivery of the product directly to the largest population of pathogens. Edrington et al. (2003b) used an experimental sodium chlorate product, designed to by-pass the rumen, and found that supplementation of the product reduced *E. coli* O157:H7 populations from the cecum and rectum of experimentally inoculated sheep.

Ascophyllum nodosum has also been studied for its potential ability to reduce pathogens in ruminant animals when supplemented in the diet. *Ascophyllum nodosum* is a type of brown seaweed, typically found in the North Atlantic basin, which has often been consumed as a source of iodine by people with an iodine deficiency. It has been shown that this brown seaweed, *Ascophyllum nodosum*, contains antimicrobial properties that can reduce the incidence of *E. coli* O157:H7 when fed to ruminants prior to harvest (Braden et al., 2004). Indeed, supplementation of *Ascophyllum nodosum* (Tasco-14) for

14 days prior to harvest significantly reduced the levels of *E. coli* O157 and *E. coli* O157:H7 in feces and on hide samples of feedlot steers when compared to controls. Additionally, cattle supplemented with Tasco-14 showed a lower prevalence of *Salmonella* immediately prior to harvest when compared to controls, although both groups showed an increase in *Salmonella* incidences from the presupplementation period (Braden et al., 2004).

Bacteriophages

The use of bacteriophage viruses has also been investigated for their potential benefits in reducing pathogen populations in the live animal in hopes of reducing subsequent carcass contamination. Bacteriophages are viruses that specifically kill bacteria. These viruses are common members of the intestinal microbial flora of food animals and it has been recently suggested that administration of bacteriophages to livestock may assist in the elimination of specific pathogens within the gastrointestinal tract (Callaway et al., 2004). In vitro studies have shown isolated bacteriophages to be successful in eliminating stains of *E. coli*, including the O157:H7 strain (Bach et al., 2002a; Callaway et al., 2002b). However, their use in the live animal has not been effective in reducing populations of *E. coli* O157:H7 when administered to experimentally inoculated sheep (Bach et al., 2002a; Callaway et al., 2002b). Additionally, bacteriophages have been ineffective against other pathogenic bacteria, including *Salmonella* and *Streptococcus* (Callaway et al., 2002b). Bacteriophages have a high degree of specificity, though, and some phages are active only against a specific strain of bacteria. Thus, the effectiveness of a bacteriophage in reducing populations of

pathogenic microorganisms may depend on the bacteriophage that is selected and the ability of the phage to recognize specific receptors on the target bacterium so that it may act against that particular strain (Callaway et al., 2003).

Hide wash

Washing cattle hides with or without antimicrobial agents, including lactic acid (0.5%) and chlorine (50 ppm) was shown to be ineffective in reducing aerobic plate counts, coliforms, *E. coli*, and *Salmonella*. In fact increased levels of contamination were seen indicating that washing may have simply released bacteria that were hidden inside of dirt, mud, and feces on the hide (Mies et al., 2004). However, application of high concentrations of lactic acid (4 to 6%), acetic acid (4 to 6%), and ethanol (100 to 400 ppm) were shown to reduce numbers of *Salmonella* Typhimurium on hide samples inoculated with the bacterium under controlled laboratory conditions. Unfortunately, these high concentrations bring about animal welfare concerns and may be potentially hazardous to human health (Mies et al., 2004).

Vaccination

The development of a vaccine that is capable of reducing pathogenic bacteria has been under recent investigation. A vaccine containing Type III secreted proteins from *E. coli* O157:H7 has been used in field trials and the results of its efficacy have been variable. Type III secreted proteins are thought to be required for intestinal colonization of *E. coli* O157:H7 in bovine animals as they do play a role in the colonization of *E. coli* O157:H7 in non-bovine hosts (Potter et al., 2004). Potter et al. (2004) found that

vaccination with Type III secreted proteins significantly reduced the number of *E. coli* O157:H7 excreted in feces and the numbers of animals excreting and the duration of excretion of *E. coli* O157:H7 by cattle in a typical feedlot setting. However, another report showed administration of the vaccine containing type III secreted proteins to feedlot cattle had no significant effect on the pen prevalence of *E. coli* O157:H7 (Van Donkersgoed et al., 2005). Effectiveness of the vaccine may be affected by the amount of time between immunizations, and the number of doses given of the vaccine, which may account for the some of the differences in results that have been reported. Further research is needed to determine if vaccination is a plausible means of reducing pathogenic populations in livestock.

ANTIBIOTICS, PROBIOTICS AND PREBIOTICS

Antibiotics

Antibiotics have long been used within the livestock industry not only for therapeutic means in treating sick animals, but also for subtherapeutic reasons aimed at improving livestock performance and maximizing production efficiency. Unfortunately, the increase in antibiotic resistance of several strains of bacteria, including potential pathogens, has led to concerns regarding the continued use of these substances. Antibiotics have been shown to decrease pathogenic bacteria, as seen in a study by Elder et al. (2002) in which oral administration of neomycin sulfate reduced the fecal excretion of *E. coli* O157:H7 in naturally infected cattle to non-detectable levels 72 hours post treatment. Loneragan and Brashears (2005) reported reductions of 98.5% and 95% in fecal and hide recovery, respectively, when neomycin sulfate was included in water for 2

days. However, there is always the potential for bacteria to eventually become resistant to any antibiotic after repeated exposure to the substance. Fears of this antibiotic resistance crossing over into human medicine have led to questions on whether subtherapeutic use of antibiotics in animal agriculture should continue. In fact, the European Union has already implemented a ban on the use of antibiotics as an ingredient in animal feed in order to boost growth in livestock.

Because of increasing fears over antimicrobial resistance, new products are currently being developed and investigated for their effectiveness on animal production efficiency and their influence on pathogenic microorganisms. It is the hope that these products will be more widely accepted among society for their use in livestock production and will enable the U.S. livestock industry to phase out the use of antibiotics without large economic complications.

Probiotics

Probiotics and prebiotics may offer an alternative to the use of antibiotics in production livestock settings. A probiotic has been defined as a live microbial food supplement that benefits the host by improving its intestinal microbial balance (Fuller, 1989). Because the term probiotic includes microbial cultures, extracts, and enzyme preparations, the term direct fed microbial (DFM) has more recently been used in place of probiotic to describe feed products that contain live naturally occurring microorganism (Yoon and Stern, 1995). The Food and Drug Administration (FDA) defines DFM as products that contain live (viable) microorganisms. Bacterial DFM often include species

of *Enterococcus*, *Streptococcus*, and *Bifidobacterium*, but the most highly used DFM is *Lactobacillus*, particularly *L. acidophillus*.

Lactobacillus spp.

It is important that DFM inhibit the growth of pathogenic bacteria without effecting normal gut microbes, and certain strains of lactic acid bacteria have been found to possess this capability (Newman et al., 1990). There are several strains of *L. acidophilus* that have been used in direct-fed microbials to reduce the prevalence of fecal shedding of *E. coli* O157 in cattle. Supplementation of *L. acidophilus* strain NP51 (also known as NP747) was shown to reduce the prevalence of *E. coli* O157 fecal excretion of cattle throughout the feeding period in a dose dependent manner, as there was a linear decrease in the prevalence of *E. coli* O157 with increasing NP51 dose (Younts-Dahl et al., 2005). These results are supported by others in which daily supplementation of *L. acidophilus* NPC 747 or *L. cristatus* NPC 750 direct-fed microbials in cattle diets decreased the fecal excretion of *E. coli* O157:H7 during the feeding period, with the NPC 747 treatment being the most effective (Brashears et al., 2003). In fact, Brashears et al. (2003) found *E. coli* O157:H7 was approximately twice as likely to be detected in cattle that were not supplemented with *L. acidophilus*. Younts-Dahl et al. (2005) also reported cattle receiving *L. acidophilus* supplementation at 10^9 CFU/steer/day were 80% less likely to be excreting detectable levels of *E. coli* O157 and 62% less likely to be carrying *E. coli* O157 on their hides at slaughter compared to controls. Differences in prevalence among feedlot pens indicate that supplementation of DFM in cattle diets may decrease environmental contamination, leading to decreases in the amount of *E. coli* O157:H7 on hide samples (Brashears et al., 2003). This is important because the hide has been shown

to be the primary source of subsequent carcass contamination (Barkocy-Gallagher et al., 2003).

DFM consisting of multiple bacterial species may further reduce the presence of *E. coli* O157:H7 in fecal and hide samples. Supplementation of a mixture of *S. faecium*, *L. acidophilus*, *L. casei*, *L. fermentum* and *L. plantarum* significantly reduced the fecal excretion of *E. coli* O157:H7 in lambs experimentally infected with *E. coli* O157:H7 (Lema et al., 2001). However, supplementing a combination of the NP51 and NP45 strains of *L. acidophilus* had no effect on fecal excretion of *E. coli* O157, rather it increased the likelihood that an animal would be excreting detectable levels of the pathogen indicating that the two strains may be antagonistic (Younts-Dahl et al., 2005).

Often *L. acidophilus* has been supplemented in combination with propionate-producing bacteria, *Propionbacterium*. Cattle supplemented with *L. acidophilus* strain NP51 in combination with *P. freudenreichii* were 57% less likely to be excreting detectable levels of *E. coli* O157 compared to those cattle that received no DFM (Younts-Dahl et al., 2004). Interestingly, cattle fed cultures of *L. acidophilus* strain NP51 in conjunction with *P. freudenreichii* also had lower prevalence of *E. coli* O157:H7 in feces than steers feed the same DFM's in addition to *L. acidophilus* strain NP45 (Elam et al., 2003). This gives further evidence that *L. acidophilus* strains NP51 and NP45 may be antagonistic to each other. However, prevalence of *E. coli* O157:H7 on hides was lower for steers fed the combination of *L. acidophilus* strains and *P. freudenreichii* than control steers and those fed only 1 strain of *L. acidophilus* and *P. freudenreichii* (Elam et al., 2003). It is important to note that hide samples often do not reflect the number of

animals that are actually infected with *E. coli* O157:H7, as environmental contamination may play a large role in hide prevalence.

Saccharomyces cerevisiae

Since FDA defines DFM as products that contain live microorganisms, DFM encompass not only bacteria, but fungi as well. *Saccharomyces cerevisiae* is a yeast that has been proposed to have an influence on the intestinal microbial population, possibly leading to improvements in digestibility, especially in terms of fiber. Supplementation of a live yeast culture of *S. cerevisiae* increased the concentration of cellulolytic and proteolytic microorganism in the rumen (Dawson et al., 1990; Yoon and Stern, 1996). However, Newbold et al. (1995) found that the ability of *S. cerevisiae* to modify the rumen bacterial population is dependent on the strain that is used. For example, *S. cerevisiae* strains NCYC 240, NCYC 1026, and Yea-Sacc (Alltech, Nicholasville) were reported to stimulate total and cellulolytic bacterial numbers in vitro and in sheep (Newbold et al., 1995). Yea-Sacc is a commercial product containing *S. cerevisiae* strain NCYC 1026. Yea-Sacc was more specifically reported to stimulate the growth of the lactate utilizing bacteria *Selenomonas ruminantium* and increase lactate uptake by this particular bacterium (Nisbet and Martin, 1991). *S. cerevisiae* NCYC 694 and NCYC 1088, however, reportedly had no affect on rumen bacterial numbers (Newbold et al., 1995). However, unlike DFM containing strains of *Lactobacillus acidophilus*, no reports have identified the effect of strains of *S. cerevisiae* on pathogenic microorganisms within the gastrointestinal tract.

Previous work by Gedek (1999) demonstrated that *S. cerevisiae*, ssp. *boulardii* have the ability to bind to enteric pathogens, including *E. coli* O157:H7 and the multi-

drug resistant *Salmonella* DT104, preventing adhesion of these pathogens to the mucosal membrane of the gut which is crucial to the virulence of these microorganisms. Levucell SB (Lallemand Animal Nutrition, Milwaukee, WI) is a feed additive that contains the CNCM I-1079 strain of *S. cerevisiae*, ssp. *boulardii*. Reports on Levucell's effect on digestibility in ruminants have been inconsistent; however, it has been shown to decrease ruminal protozoal counts (Arcos-Garcia et al., 2000; Garcia et al., 2000). This is important because ciliated protozoal populations prey on bacteria (Beharka and Nagaraja, 1993). Therefore, a decrease in the protozoal population would be expected to cause an increase in the number of rumen bacteria. These bacteria could not only lead to increases in digestibility, but could also compete with pathogenic bacteria for adhesion to the mucous membrane of the gut. This attachment is essential for colonization and proliferation of pathogens and by preventing this critical step of the infection process through competition by rumen bacteria it may be possible to eliminate these pathogens from the gastrointestinal tract. However, the use of Levucell SB to reduce pathogens in cattle pre-harvest has not been previously reported.

Additional benefits of probiotics

In addition to the ability of DFM to reduce pathogenic prevalence in and among live animals there is evidence that these products may also positively influence animal performance and feed efficiency. This would make DFM ideal as a replacement to antimicrobial usage in livestock production. It has been shown that DFM improved average daily gain and feed efficiency, in conjunction with reducing fecal shedding of *E.coli* O157:H7 (Lema et al. 2001). Krehbiel et. al. (2003) suggested that the inhibition of methane production by *Lactobacillus* species may promote *Propionibacterium* to

produce proprionate thereby accounting for the improvement in energy efficiency and animal performance that has been reported when feeding DFM. Kvietkute et al. (2005) reported Levucell SB to significantly increase the growth rate of experimental pigs, but its impact on ruminant animals has not been studied. This combination of a reduction in pathogenic bacteria together with enhancements in performance would make DFM ideal as a replacement to antimicrobial usage in livestock production.

Prebiotics

Mode of Action

Although the use of probiotics, or direct-fed microbials, has been proven to reduce *E. coli* O157:H7 and possibly improve animal performance in livestock, there is still concern about the idea of adding live microorganisms to animal feed. Thus, there has been increased focus on the use of prebiotics. Prebiotics are nondigestible nutritional compounds that selectively stimulate the growth of endogenous lactic acid bacteria and *Bifidobacteria* to improve the health of the host (Walker and Duffy, 1998). In contrast to supplementing live microorganisms to increase the population of beneficial microflora in the gastrointestinal tract, as in probiotics, compounds are fed that promote the growth of those beneficial microorganisms that already exist within the gastrointestinal tract of the host. Bacteria fed a preferential food substrate, such as a prebiotic, are thought to be more likely to have a proliferative advantage over other bacteria, including those that may be pathogenic (Walker and Duffy, 1998).

In humans, prebiotics include nondigestible oligosaccharides, such as fructooligosacchaarides, that resist hydrolysis in the upper gastrointestinal tract, thereby

reaching the colon where they are fermented by symbiotic bacteria such as *Bifidobacteria* and lactic acid bacteria (Walker and Duffy, 1998). Requirements for a food ingredient to be classified as a prebiotic include: 1) must neither be hydrolyzed nor absorbed in the upper part of the gastrointestinal tract; 2) must be a selective substrate for one or a limited number of potentially beneficial commensual bacteria in the colon; and 3) must be able as a consequence to alter the colonic microflora toward a more desirable (healthier) composition (Collins and Gibson, 1999). Consumers in Japan and Europe are already aware of the health benefits provided by consuming prebiotics and the importance of these products is slowly becoming accepted by Americans. Consumption of prebiotics by humans has been shown to have many health benefits from enhancement of mineral absorption to prevention of colon carcinogenesis (Brannon, 2003). In ruminants, fermentation of prebiotics within the colon results in the release of short-chain fatty acids, propionate, acetate, and butyrate, which decrease the pH of the colon causing an acidic environment, which may be detrimental to pathogenic bacteria but favorable for beneficial bacteria (Brannon, 2003).

Amaferm

Amaferm® (Biozyme Inc., St. Joseph, MO) is a feed supplement that may have the ability to act as a prebiotic in ruminant livestock. Amaferm is a fermentation extract produced from a select strain of *Aspergillus oryzae*, which works to stimulate the number and activity of the endogenous microflora. Amaferm is thought to have two modes of action; it increases endogenous populations of ruminal bacteria and it increases the growth rate and activity of rumen fungi (Beharka and Nagaraja, 1998; Chang et al., 1999).

Supplementation of Amaferm has been shown to increase populations of ruminal pectinolytic and hemicellulolytic bacteria and cause higher counts of amylolytic and cellulolytic bacteria (Beharka et al., 1991). Similarly, Beharka and Nagaraja (1998) found that Amaferm increased the growth rate of certain bacteria that are known to digest fiber or utilize lactate. Fiber digesting species that were stimulated in the presence of Amaferm in vitro included *Rumminococcus albus* and *Fibrobacter succinogenes* (Beharka and Nagaraja, 1998). Increases in digestibility have been associated with Amaferm supplementation and it has been proposed that this increase in fiber digestibility is due to Amaferm stimulation of bacterial activity (Beharka and Nagaraja, 1993). This increase in fiber digestibility may ultimately lead to increases in animal performance.

Those species that utilize lactate in which Amaferm has been reported to have an effect upon include *Megasphaera elsdenii*, *Selenomonas lactilytica*, and *Selenomonas ruminantium* (Beharaka and Nagaraja, 1998). The stimulation of lactate utilizing bacteria, in turn, leads to an increase in lactate utilization within the rumen (Nisbet and Martin, 1990; Waldrip and Martin, 1993). The increase in lactate utilization by rumen bacterial species hinders the post-feeding decline in ruminal pH (Nisbet and Martin, 1990). Low ruminal pH is typically associated with acidosis which can result in reduced feed intake and performance by the animal (Owens et al., 1998). Thus in summary, supplementation of Amaferm stabilizes ruminal pH via an increase in lactate utilizing bacteria and a subsequent increase in lactate uptake, which prevents alterations in rumen function. Moreover, Amaferm may potentially impact the presence of pathogenic microorganism by increasing the number of competing beneficial bacteria along the gastrointestinal tract. However, it remains unclear as to whether or not the rumen, the

primary location in which AMF has been reported to have an effect, serves as a reservoir for colonized *E. coli* O157:H7 and *Salmonella* spp. Brown et al. (1997) reported the forestomach as the primary site of *E. coli* O157:H7 localization and proliferation. However, a recent study by Naylor et al. (2003) indicates that the lymphoid follicle-dense mucosa at the terminal rectum (mucosal epithelium within a defined region extending up to the last 5 cm of the rectal-anal junction) is the principal site for colonization of *E. coli* O157:H7 in cattle. Whether or not Amaferm impacts the microbial population in this area of the gastrointestinal tract is unknown. Furthermore, no studies have analyzed the impact of Amaferm on the presence of *E. coli* O157:H7 or *Salmonella* in ruminant animals.

Amaferm not only influences rumen bacteria, but has also been shown to increase the growth of rumen fungi (Harper et al., 1996; Chang et al., 1999). Rumen fungi are known to be the primary digesters of lignin, the nondigestible portion of plants that often limits the extent to which plant tissue can be degraded (Akin, 1986). Rumen fungi are capable of penetrating the cuticle of the lignocellulosic complex, breaking these bonds and allowing fiber degrading bacteria to adhere to the inner surface of the plant (Akin and Borneman, 1990). Additionally, rumen fungi produce high amounts of fiber degrading enzymes including cellulases, hemicellulases, and xylanases (Akin and Borneman, 1990). Harper et al. (1996) demonstrated, in vitro, the ability of Amaferm to stimulate the growth rate, cellulase secretion, and fungal mass of three predominant rumen fungi: *Neocallimastix frontalis* EB 188, *Piromyces communis* DC 193, and *Orpinomyces* spp. Schmidt et al. (2004) further demonstrated that Amaferm accelerated the production and

maturation of zoospores of the fungi *Neocallimastix frontalis* EB 188 and elevated the levels of several enzymes including cellulase.

The increase in fermentable fiber that is provided via stimulation of rumen fungi, leads to the production of short chain fatty acids. Martin and Nisbet (1990) suggest that Amaferm influences digestive microflora by providing additional unidentified growth factors to ruminant microorganisms as observed by increases in the production of most fermentation products, such as short-chained fatty acids. These short chain fatty acids are the preferred energy source for colonic epithelial cells, and via metabolism they decrease the pH level in the intestinal lumen, establishing an acidic environment that is favorable for beneficial bacteria but detrimental to the survival of pathogenic bacteria (Swanson et al., 2002; Brannon, 2003). However, limited information is available on whether or not Amaferm is capable of decreasing the number of pathogenic microorganisms within ruminant animals.

CHAPTER 3

EFFECTS OF AMAFERM SUPPLEMENTATION TO CONCENTRATE OR FORAGE BASED DIETS ON GROWTH PERFORMANCE AND CARCASS CHARCTERISTICS OF FINISHING LAMBS

ABSTRACT

Ninety-six Dorset x Hampshire lambs (initial weight range 22.7 to 34.0 kg) were used in a complete block experiment with a 2 x 2 factorial arrangement of treatments to determine the effects of Amaferm® (**AMF**) supplementation in diets containing either a high proportion of starch (high concentrate; **CON**) or high proportion of fiber (high forage; **FOR**) on growth performance and carcass characteristics. Lambs were allotted to 24 pens (four lambs per pen) that were blocked by sex and weight. Twelve pens of lambs received the FOR diet and 12 pens were fed the CON diet. Within each diet treatment group, six pens received AMF. Lambs were fed until the average live weight of each pen reached the target weight (55.4 kg for wethers and 50 kg for ewes), at which time the entire pen of lambs was harvested. Weight off test was included in the model as a covariate for the analysis of carcass measurements. Lambs that received the CON diet consumed less ($P < 0.05$) average DMI (1.42 vs. 1.93 kg) and produced carcasses that had greater ($P < 0.05$) backfat (0.80 vs. 0.65 cm), body wall (2.36 vs. 1.95 cm), and ribeye area (15.13 vs. 13.31 cm^2). Additionally, the CON diet resulted in carcasses that

received higher ($P < 0.05$) leg (12.23 vs. 11.37) and conformation (11.96 vs. 11.37) scores, as well as higher ($P < 0.05$) quality grades (12.46 vs. 11.67). Supplementation of AMF had no effect ($P < 0.05$) on carcass characteristics. The results of feedlot performance indicated that an interaction occurred between energy source and feed supplement ($P < 0.05$). For lambs that received the CON diet, AMF supplementation resulted in improved ($P < 0.05$) feed efficiency (0.257 vs. 0.245 kg gain/kg feed) and greater ($P < 0.05$) ADG (0.37 vs. 0.35 kg/d), however, it did not ($P > 0.05$) impact days on feed needed to reach the target end point (73 vs. 70 d). For lambs that received the FOR diet, AMF supplementation resulted in decreased ($P < 0.05$) feed efficiency (0.122 vs. 0.135 kg gain/kg feed), and ADG (0.23 vs. 0.26 kg/d), and resulted in a greater ($P < 0.05$) number of days on feed to reach the targeted market endpoint (106 vs. 97 d). Results indicate that at the levels fed, Amaferm® may improve growth performance for lambs finished on high concentrate diets, but not high forage diets.

INTRODUCTION

Consumer preferences have stimulated an increase in all-natural programs within the meat and livestock industries. This increase stems from a growing concern among consumers in regard to the amount of growth promoting antibiotics and exogenous hormones used in livestock production. The use of fungal supplements may offer a natural alternative to these traditional growth promotents. These products have been shown to manipulate the rumen environment in many ways that may potentially benefit the ruminant animal and lead to enhancements in production (Beharka and Nagaraja, 1993; Yoon and Stern, 1996; Schmidt et al., 2004). Amaferm (**AMF**; BioZyme, Inc., St.

Joseph, MO) is one such product that is a fermentation extract of the mold *Aspergillus oryzae*. AMF is thought to have two modes of action; it increases endogenous populations of ruminal bacteria and it increases the growth rate and activity of rumen fungi (Beharka and Nagaraja, 1998; Harper et al., 1996).

Through the acceleration of rumen fungi and fiber digesting bacteria, AMF has the potential to increase fiber digestibility. Rumen fungi are capable of penetrating the cuticle of the lignocellulosic complex of plants, breaking these bonds and allowing fiber degrading bacteria to adhere to the inner surface of the plant tissue (Akin and Borneman, 1990). AMF has been shown to increase NDF and ADF degradability; however, the effects of extract addition are dependent on forage type (Gomez-Alarcon et al., 1990; Beharka and Nagaraja, 1993).

Moreover, through the increase in lactate utilizing bacteria such as *Megasphaera elsdenii* and *Selenomonas ruminantium*, AMF has been reported to increase lactate utilization within the rumen which may hinder the post-feeding decline in ruminal pH (Nisbet and Martin, 1990; Waldrip and Martin, 1993). Low ruminal pH is typically associated with acidosis which can result in reduced feed intake and performance by the animal (Owens et al., 1998). Unfortunately, previous research has not looked specifically at the effects of AMF on animal performance and carcass characteristics. Thus, the objective of this study was to examine the effects of AMF with two dietary energy sources on growth performance and carcass characteristics of feedlot lambs.

MATERIALS AND METHODS

Animals and Diets

Ninety-six Dorset x Hampshire lambs (initial weight range 22.7 to 34.0 kg) were used in a randomized complete block experiment with a 2 x 2 factorial arrangement of treatments to determine the effects of Amaferm addition to diets containing either a high proportion of starch (concentrate diet) or cellulose (forage diet) on animal performance and carcass characteristics. Lambs were housed at the sheep feedlot facility located at the Ohio Agriculture Research and Development Center in Wooster, OH. All pens were constructed using expanded metal floors with metal gates on three sides and a wooden fence line feed bunk on the fourth side. Pens were 1.49 x 4.88 m with 1.49 m of bunk space. Each pen had an automatic water cup so that water was available at all times. There were forty-eight wethers and forty-eight ewes used in the experiment, which were allotted to pens based on sex and initial weight. Each pen contained 4 lambs for a total of 24 pens. Lambs were individually weighed, ear tagged, and vaccinated against internal and external parasites with injectable Ivermectin. Initial and final weights of the lambs were determined using the average of weights taken on two consecutive days.

Diet compositions are shown in Table 3.1 and were formulated to meet the dietary nutrient requirements for lambs (NRC, 1985). Lambs were restricted fed during the first two weeks of the trial such that lambs fed the concentrate diet received feed at a rate of 3.5% of their live body weight, and lambs fed the forage diet received feed at a rate of 4.5% of their live body weight, based on the average weight of the lambs in each pen. Feed intake was restricted to allow animals to adapt to the change in diet and prevent the occurrence of digestive disorders. Following the two week adaptation period, lambs were

fed ad libitum for the remainder of the trial. The concentrate diet contained ground corn, alfalfa, soybean hulls, and soybean meal to provide 2.0 Mcal /g NE_m and 1.4 Mcal/g NE_g. The forage diet was composed of alfalfa, soybean hulls, and soybean meal to supply 1.4 Mcal/g NE_m and 0.82 Mcal/g NE_g. Amaferm was added to each of the two diets to provide 0 or 1g per head per day. All feed was in pelleted form including the Amaferm supplement. The forage diet was composed of 52.84% NDF and the concentrate diet was composed of 21.8% NDF. Feed offered and feed refused was weighed daily in each pen prior to refeeding at 0830. Because sorting was expected, feed was not allowed to remain in the feed bunk for more than 1 day before being discarded. Pens of lambs never had intake increased or decreased by more than 10% of the previous day's intake.

Performance Measurements

Initial and final weights were calculated as the average of weights taken on two consecutive days at the start and finish of the trial, and interim weights were taken every 14 days prior to feeding at 0800. Average daily gain (ADG), dry matter intake (DMI), feed efficiency (kg gain/kg feed), and days required to reach harvest weight were determined for all lambs. Lambs were removed from the trial, on a pen basis, as each pen reached the predetermined market weight range of 49.9 kg to 54.4 kg for ewes and 54.4 kg to 59.0 kg for wethers.

Carcass Measurements

Once the average weight of each pen reached the target market weight, lambs were transported to The Ohio State University Meat Science Laboratory in Columbus,

OH so that all lambs in the pen could be harvested. Hot carcass weights were recorded immediately prior to chilling. Backfat thickness, body wall thickness, and ribeye area were measured at the 12th-rib after carcasses had been chilled for 48 h. Leg, confirmation, and lean quality scores as well as marbling and quality grades were subjectively determined for each carcass by an experienced evaluator 48 h after harvest. The longissimus muscle from the 11th to 12th rib was removed from one side of each carcass, trimmed of external fat, ground and subsampled for determination of moisture and ether-extractable lipid (AOAC, 1984).

Statistical Analysis

The experiment was designed as a randomized complete block design with a 2 x 2 factorial arrangement of treatments with the main effects of energy source and amount of Amaferm supplementation to evaluate factors associated with performance and carcass characteristics. Lambs were initially blocked by weight (light, medium, or heavy) and by gender (ewe or wether). Data were analyzed using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. Performance and carcass data were analyzed using a model that included gender, weight, energy source, Amaferm supplementation (0 vs. 1 g), and the energy source x level of Amaferm supplementation interaction. Additionally, weight off test was included in the model as a covariate for carcass measurements. Treatment means were compared with Fisher's protected LSD using the PDIFF statement of SAS (1999) when protected by a significant ($P < 0.05$) F-value.

RESULTS AND DISCUSSION

Feedlot Performance

Initial and final body weights of lambs (Table 3.2) were similar for all treatments ($P > 0.05$). Average daily feed intake for those lambs fed the forage diet was greater than that of lambs fed the concentrate diet ($P < 0.01$). This is expected because the lambs were eating to meet their net energy requirements and the forage diet contained less net energy than the concentrate diet (1.388 vs. 2.038 NE_m).

The results for ADG, days on feed, and feed efficiency indicated that an interaction occurred between energy source and feed supplement ($P < 0.05$). AMF supplementation led to a 6% increase ($P < 0.05$) in ADG (0.37 vs. 0.35 kg/d) in lambs fed the concentrate diet. However, in lambs fed the forage diet AMF decreased ($P < 0.05$) ADG (0.23 vs. 0.26 kg/d) by 12%. Because AMF did not have a significant impact ($P = 0.84$) on feed intake among concentrate or forage-fed lambs, AMF ultimately resulted in a 5% increase in feed efficiency ($P < 0.05$) in lambs fed the concentrate diet and a 10% decrease in feed efficiency ($P < 0.05$) in lambs fed the forage diet. Furthermore, because AMF decreased ADG in forage fed lambs, those lambs required more days on feed to reach the target market endpoint ($P < 0.05$). AMF had no effect on the number of days needed to reach market weight in those lambs fed the concentrate diet ($P = 0.31$).

Feeding high levels of grain to cattle has been historically associated with a decrease in the pH of the rumen. This results from fermentation of carbohydrates by anaerobic microbes in the rumen that leads to the production of volatile fatty acids. Ingestion of high amounts of concentrates also provides the substrate for rapid proliferation of bacteria such as *Streptococcus bovis* and *Lactobacillus* species that

produce large amounts of lactate from carbohydrates which can lead to a decrease in rumen pH (Hungate et al., 1952; Slyter, 1976). Low ruminal pH is typically associated with acidosis which can result in reduced feed intake and performance by the animal (Owens et al., 1998). AMF has been reported to stimulate the growth rate of bacterial species that are capable of utilizing lactate and thus preventing the decline in rumen pH (Beharka and Nagaraja, 1998). Nisbet and Martin (1990) demonstrated that AMF stimulated the growth rate of the bacterium *Selenomonas ruminantium* and increased the utilization of lactate by this bacterium. Moreover, Waldrip and Martin (1993) showed an increase in the growth rate of the predominate lactate-utilizing bacterium *Megasphaera elsdenii* in the presence of AMF and showed an increase in the uptake of lactate as well. The increase in lactate utilization by these rumen bacterial species hinders the post-feeding decline in ruminal pH that is seen when high amounts of concentrates are consumed by the animal (Nisbet and Martin, 1990). In the present study, although neither ruminal pH nor the presence of lactate-utilizing bacteria was measured, AMF may have stabilized ruminal pH, thereby preventing any digestive disturbances that could have occurred with the feeding of high levels of concentrates. This stabilization of ruminal pH may have contributed to the improvement in feed efficiency that was observed in lambs fed the concentrate diet supplemented with AMF compared to lambs fed the concentrate without AMF supplementation.

In the present study, daily gain and feed efficiency were decreased when AMF was supplemented in the diets of lambs fed the forage diet. AMF has been shown to increase fiber digesting species of bacteria in vitro and in young calves (Beharka et al., 1991; Beharka and Nagarja, 1998). In fact, AMF was shown to increase the growth rate

of the bacteria *Fibrobacter succinogenes* and *Ruminococcus albus*, which are among the predominat fibrolytic bacteria in the rumens of cattle and sheep (Dehority et al, 1991; Beharka and Nagaraja, 1998). Furthermore, AMF has been shown to stimulate the growth and activity of rumen fungi by accelerating the production and maturation of zoospores, thereby giving it two modes of action (Chang et al., 1999; Schmidt et al., 2004). The increase in rumen fungi is important because ruminant feedstuffs, particularly forage tissues, are protected by a cuticle that is extremely resistant to bacterial attachment (Akin, 1989). The hyphae of rumen fungi, however, have the unique ability to directly penetrate this cuticle of the lignocellulosic complex, breaking these bonds and allowing fiber degrading bacteria to access and adhere to the inner surface of the plant (Akin and Rigsby, 1987; Akin and Borneman, 1990). Rumen fungi also produce high amounts of fiber degrading enzymes including cellulases, hemicellulases, and xylanases that are secreted from their growing hyphae tips (Akin and Borneman, 1990). Schmidt et al. (2004) reported elevated levels of several of these enzymes including cellulase when AMF was added to fungal cultures.

Because of the increase in both rumen fungi and fiber digesting bacteria that have been reported with AMF supplementation, it would be expected that there would be an increase in fiber degradation which may cause enhancements in animal performance. Reports on the effect of AMF on digestibility, however, have been inconsistent. For instance AMF was shown to stimulate ADF and NDF degradation of alfalfa, bromegrass, and high endophyte fescue, but had no effect on fiber degradation of pure cellulose, corn silage, low endophyte fescue, prairie hay, or wheat straw in vitro (Beharka and Nagaraja, 1993). These results demonstrate that the effects of AMF maybe dependent on forage

type. Additionally, AMF was found to have no effect on the rate or extent of fiber degradation in situ when bromegrass or alfalfa was fed, however, the proportion of *Ruminoccocus albus* isolates was increased when AMF was supplemented with bromegrass (Varel and Kreikemeier, 1994). In the current study the effect of AMF on rumen fiber digesting bacteria or fiber degradation was not measured, but AMF was found to have a negative impact on feedlot performance. Recall that all feeds in the current study were fed in pelleted form, whereas most previous studies have looked at the effects of AMF on long-stemmed forages, which may partially explain the observations seen in this study. It has been recognized that in order for fiber digestion to occur in the rumen, fiber digesting bacteria must first attach to feed particles as they make their passage through the rumen. This attachment serves to appose digestive enzymes with their specific substrates, thereby concentrating digestion within a small area (Chesson and Forsberg, 1988). However, because of the reduced particle size of the pelleted forage fed in this study, the rate of passage of the feed particles from the rumen may have been too fast to allow the microbes to attach, penetrate, and digest the feedstuffs (McAllister et al., 1994). Thus, although there may have been an increase in the amount of fiber digesting bacteria within the rumen from the addition of AMF, the speed with which the feed particles exited the rumen may have been too quick for these microorganisms to impact fiber digestion. Additionally, it is possible that the fungi within the rumen may not have been affected by AMF supplementation, as reported in other studies, because of the increased rate of passage of feed particles. Prolonged residence in the rumen is necessary for rumen fungi because of their long generation times, and the populations of these microorganisms are rapidly depleted if they are unable to attach to feed particles and

delay their passage from the rumen (McAllister et al., 1994). Therefore, although AMF may have had the potential to increase the population of fungi within the rumen, the type of feed used in the experiment may have actually decreased the population of rumen fungi by shortening their time within the rumen and not allowing them the time needed to replicate.

These explanations offer reason as to why AMF did not increase animal performance in those lambs fed the pelleted forage diet, but do not explain why AMF decreased performance. From this rationale it would be expected that those lambs on the forage diet that were supplemented with AMF would have performed similarly to those lambs fed the forage diet that were not supplemented with AMF. Further research is needed to explain why AMF has a negative impact on feedlot performance in lambs fed a ground, pelleted forage diet. However, one hypothesis is that AMF may alter the population of microorganisms to one that is less capable of digesting a ground pelleted feed composed primarily of alfalfa and soybean hulls.

Carcass Characteristics

Weight off test was used as a covariate in the analysis of all carcass measurements and did not differ among treatments ($P > 0.05$). No interactions between energy source and treatment were observed; therefore the main effects are presented in Table 3.2. Supplementation of AMF had no significant effect ($P > 0.05$) on any carcass characteristics measured in the study. Energy source, however, did have an impact on carcass characteristics. Concentrate fed lambs showed increases ($P < 0.05$) in 12^{th}-rib LM area, backfat thickness, and bodywall thickness compared to lambs fed the forage

diet. Additionally, lambs fed the concentrate diet had higher leg ($P < 0.05$) and confirmation ($P = 0.04$) scores and higher quality grades ($P < 0.05$). There were no differences in HCW ($P = 0.42$), dressing percent ($P = 0.53$), lean quality score ($P = 0.64$), marbling ($P = 0.10$), or chemical intramuscular fat ($P = 0.83$) due to differences in energy source. Fluharty et al. (1999) reported increases in 12th-rib loin eye area in lambs fed a concentrate diet compared to lambs that grazed alfalfa. The maintenance energy requirements of organs are affected by the level of nutrition (Ferrell et al., 1986). Fluharty et al. (1999) attributed the smaller loin eye area of lambs grazing alfalfa to the increase in energy and protein requirements of other tissues and visceral organs compared to striated muscle tissue within these forage fed animals. In addition to 12th-rib LM area, backfat and body wall thickness were also greater for lambs fed the concentrate diet. Similar results were reported by Murphy et al. (1994) in which greater quantities of total fat were observed in each of the three major fat depot sites (subcutaneous, seam, and mesenteric) in lambs fed concentrates at some time during the finishing period compared to lambs finished solely on alfalfa.

IMPLICATIONS

AMF supplementation at 1 g per head per day throughout the finishing period could be used to increase ADG and improve feed efficiency in lambs fed a concentrate diet. However, AMF was found to have a negative impact on feedlot performance when lambs were finished on a forage diet. In lambs fed the concentrate diet, AMF was found to increase ADG by 6% and improve feed efficiency by 5%, with no change in feed intake. For comparison reasons, in a review by Goodrich et al. (1984) it was reported

that supplementation of monensin, a commonly used ionophore, to cattle diets results in a 1.6% increase in ADG, a 6.4% reduction in feed intake, and a 7.5% improvement in feed efficiency, with considerable variability in response. Lasalocid, another ionophore commonly fed to sheep, was shown to improve feed utilization by 6% (Couvaras, 1980). However, the use of ionophores is prohibited in production systems that claim to be producing 'all-natural' products. Further research is needed to determine if it would be beneficial to use AMF as an alternative to or in conjunction with traditional growth-promoting antibiotics (ionophores).

Item	Forage[ab]	Concentrate[ab]
Ingredient	——% DM basis——	
Corn	.000	68.789
Alfalfa	54.701	4.749
Soybean hulls	38.207	9.603
Soybean meal	5.300	7.573
Corn gluten meal	.000	6.873
Urea	.366	.699
Dicalcium phosphate	.457	.000
Limestone	.000	.786
TM Sheep salt	.457	.437
Amonium Chloride	.366	.350
Vitamin A, 30,000 IU/kg	.009	.009
Vitamin D, 3,000 IU/kg	.009	.009
Vitamin E, 44,000 IU/kg	.046	.044
Vitavet selenium, 201 mg/kg	.082	.079
Nutrient composition		
Crude Protein, %	19.311	19.327
NDF, %	52.84	21.80
Calcium, %	1.149	.475
Phosphorus, %	.350	.362
Potassium, %	2.020	.685
Calculated NE_m, Mcal/kg	1.388	2.038
Calculated NE_g, Mcal/kg	.821	1.386

[a]Amaferm was supplemented to provide 0 or 1 g per head per day

[b]Feed rations were fed in pelleted form.

Table 3.1: Ingredient and nutrient composition of diets.

	Dietary Treatment[a]					P-values		
Item	For, C	For, AMF[b]	Con, C	Con, AMF[b]	SEM	Diet[c]	Supplement	Diet x Supplement
Initial BW, kg	29.38	29.34	29.49	29.38	.14	.5886	.62	.82
Final BW, kg	54.64	54.18	54.83	54.79	.5	.4275	.63	.68
ADFI, kg	1.95^{w}	1.92^{w}	1.41^{x}	1.42^{x}	.02	<.0001	.45	.31
ADG, kg	.26^{w}	.23^{x}	.35^{y}	.37^{z}	.01	<.0001	.55	.002
Gain/Feed, kg/kg	.135^{w}	.122^{x}	.245^{y}	.257^{z}	.003	<.0001	.95	.002
Days on Feed	97^{w}	106^{x}	73^{y}	70^{y}	2.35	<.0001	.22	.01

[a]For = forage diet; Con = concentrate diet; C = control; AMF = Amaferm

[b]Amaferm supplemented to provide 1 g per head per day

[w,x,y,z]Within a row, means with different superscripts differ (P < .05)

Table 3.2: Effect of Amaferm supplementation with two dietary energy sources on lamb performance.

	Diet[a]			Supplement[b]			
Item	Forage	Concentrate	P-value	No Amaferm	Amaferm[c]	P-value	SEM
HCW, kg[d]	27.3	28.1	.42	28.00	27.36	.55	.74
Dress %[d]	55.9	55.0	.53	56.25	54.68	.28	.98
LM area, cm^2	13.31	15.13	.0002	13.98	14.46	.21	.26
Backfat thickness, cm	.65	.80	.002	.69	.75	.17	.03
Body wall thickness, cm	1.95	2.36	<.0001	2.16	2.15	.90	.05
Leg score[e]	11.37	12.23	.003	11.70	11.91	.39	.17
Confirmation score[e]	11.37	11.96	.04	11.63	11.70	.77	.19
Lean quality score[f]	12.86	12.75	.64	12.81	12.81	1.00	.16
Marbling[g]	534.3	565.2	.10	558.26	541.25	.35	12.5
Intramuscular fat[h], %	3.91	2.97	.83	4.15	3.73	.14	.19
Quality Grade[e]	11.67	12.46	.002	12.08	12.05	.88	.14

[a] Forage diet composed mostly of alfalfa and soybean hulls; Concentrate diet composed mostly of ground corn and soybean hulls.
[b] Amaferm supplement was provided by Biozyme Inc. (St. Joseph, MO) and was completely pelleted.
[c] Amaferm supplemented to diet to provide 1 g per head per day.
[d] Standard errors for HCW and dressing percent represent a pooled average because of differences due to missing values.
[e] Leg score, confirmation score, and quality grade based on a numeric scale of 10 = low choice; 11 = average choice; 12 = high choice; 13 = prime.
[f] Lean quality score objectively measured on texture, firmness, and marbling of cut surface and based on a numeric scale of 10 = low choice; 11 = average choice; 12 = high choice; 13 = low prime.
[g] Marbling based on a numeric scale of 500-599 = low prime.
[h] Determined by ether extraction.

Table 3.3: Influence of dietary energy source and Amaferm supplementation on carcass characteristics of feedlot lambs.

CHAPTER 4

EFFECTS OF NATURAL FEED SUPPLEMENTS ON GROWTH PERFORMANCE, CARCASS CHARACTERISTICS, AND THE FECAL EXCRETION OF *E. COLI* O157:H7 AND *SALMONELLA* SPP. OF FINISHING BEEF STEERS

ABSTRACT

One hundred sixty-eight crossbred beef steers, initially weighing 250 to 340 kg, were used in a trial with a 3 x 2 factorial arrangement of treatments. The study was designed to examine the effects of natural feed supplements (*Saccaromyces cervisiae boulardii* CNCM 1079-Levucell SB (**LEV**), and *Aspergillus oryzae*-Amaferm ® (**AMF**)) with two dietary energy sources (dry whole shelled corn (**DWSC**), or high moisture corn (**HMC**)) on growth performance, carcass characteristics and the ability of these products to reduce fecal excretion of *E. coli* 0157:H7 and *Salmonella* spp. of fed cattle.

Cattle receiving HMC showed an improvement ($P < 0.05$) in feed efficiency compared to those cattle being fed DWSC (0.212 vs. 0.202 kg gain/kg feed). Addition of LEV to the high concentrate corn-based diets fed in this study did not have an impact on growth performance of feedlot steers. However, addition of AMF to a high-concentrate diet composed of DWSC resulted in an improvement ($P < 0.05$) in feed efficiency (0.208 vs. 0.194 kg gain/kg feed). Neither of the feed additives, LEV or AMF, had an affect on

carcass characteristics, nor did they reduce the incidence or apparent excretion of *E. coli* O157:H7.

No animals tested positive for *Salmonella* throughout the feeding period. A spike in the fecal excretion of *E. coli* O157:H7 was seen approximately 6 weeks after the start of the trial. Differences in the excretion of *E. coli* O157:H7 by feedlot cattle were observed between the two corn sources fed in this study. Cattle being fed HMC appeared to have more resilience against infection of *E. coli* O157:H7 as the percent of animals within a pen excreting *E. coli* O157:H7 for pens receiving HMC declined to levels near those seen prior to the peak; whereas, the average percent of cattle within a pen for those pens on the DWSC diet was greater for several weeks after the peak in fecal excretion occurred. Additionally, steers fed HMC excreted *E. coli* O157:H7 less frequently than steers fed DWSC. This study provides evidence that cattle being finished on a high concentrate diet that utilizes high-moisture corn may be more resilient to *E. coli* O157:H7 and may express a lower level of excreting *E. coli* O157:H7 than those cattle being finished on a high concentrate diet formulated with dry, whole shelled corn.

INTRODUCTION

The safety of the U.S. meat supply has become an issue at the forefront of the industry in recent years. Although most fears are focused on the global spread of foreign animal diseases such as Avian Influenza and Bovine Spongiform Encephalopathy, pathogenic microorganisms continue to be the greatest cause of food-borne illnesses. The presence of these pathogens on carcasses and the resultant meat products is not new. The meat industry has worked for years to combat the survival of *E. coli* O157:H7,

Salmonella spp., *Campylobacter* spp., and other harmful bacteria on meat products by implementing strategies after harvest to reduce or eliminate such pathogens. However, the likely reason for the presence of such pathogens on meat products is that these microorganisms are often transiently found in the gastrointestinal tract of livestock species (Meyer-Broseta et al., 2001; Jay et al., 2005). Thus, carcass contamination will likely continue to occur unless strategies are developed to reduce the presence of pathogenic bacteria in the animal itself.

Interestingly, since the U.S. FDA approved the use of ionophores as feed supplements in the mid-1970's, increased attention has been given to the relationship between initial ionophore use and the increase in *E. coli* O157:H7 food-borne illness in the U.S. (Rasmussen et al., 1999). Ionophores are a class of antibiotics known to improve feed efficiency and animal performance and are commonly fed in the diet of ruminant animals (Goodrich et al., 1984). Although many researchers have found short-term feeding of ionophores to have little effects on *E. coli* O157:H7 or *Salmonella* excretion in ruminant feces, consumer concerns over the use of antibiotics in production livestock continues to grow because of the increase in antimicrobial resistance that has spiked in recent years (Bach et al., 2002b; Edrington et al., 2003a; Van Baale et al., 2004).

Some have proposed the use of natural feed supplements, such as probiotics and prebiotics, as an alternative to antimicrobial use in livestock. These compounds have been shown by many researchers to selectively increase populations of beneficial microorganisms within the rumen which could potentially lead to improvements in animal performance similar to those seen by ionophores and other feed supplements

(Newbold, 1995; Yoon, 1996; Harper et al., 1996; Beharka and Nagaraja, 1998; Krehbiel et al., 2003). Additionally, through the stimulation of beneficial bacteria, these compounds may be able to reduce populations of pathogenic microorganisms by out competing pathogens for nutrients and places of attachment within the gastrointestinal tract. Many researchers have reported an increase in the population of fiber digesting and lactate utilizing bacteria, as well as an increase in the population of rumen fungi when the feed supplement Amaferm, a fermentation extract of the mold *Aspergillus oryzae*, was fed in ruminant diets (Gomez-Alarcon et al., 1990; Beharka, et al., 1991; Beharka and Nagaraja, 1993; Harper et al., 1996; Beharka and Nagaraja, 1998; Chang et al., 1999). Moreover, Gedek et al. (1999) reported the ability of the yeast *Saccharomyces cervisiae* ssp. *boulardii* to irreversibly bind *E. coli* O157:H7 and *Salmonella Typhimurium* DT104 to its outer membrane. Levucell SB is a feed supplement that contains the CNCM I-1079 strain of *Saccharomyces cervisiae*, ssp. *boulardii* and may have the ability to prevent adhesion of these pathogens to tissue surfaces and facilitate the removal of these microorganisms from the gastrointestinal tract.

In U.S. feedlots, cattle are finished using a variety of energy sources and diet formulations. Therefore, the objective of this study was to evaluate the efficacy of natural feed supplements (Amaferm or Levucell SB) to 1) improve feedlot performance and carcass merit, and 2) reduce the prevalence of *E. coli* O157:H7 and *Salmonella* spp. in fed cattle finished on diets containing either dry whole shelled corn or high-moisture corn.

MATERIALS AND METHODS

Animals and Treatments

One hundred sixty-eight crossbred beef steers, initially weighing 250 to 340 kg, were used in a trial with a 3 x 2 factorial arrangement of treatments. The study was designed to examine the effects of natural feed supplements (*Saccaromyces cervisiae boulardii* CNCM 1079-Levucell SB and *Aspergillus oryzae*-Amaferm ®) with two dietary energy sources on growth performance, carcass characteristics and the ability of these products to reduce fecal excretion of *E. coli* 0157:H7 and *Salmonella* spp. of fed cattle. A portion of the cattle arrived at the feedlot on October 5, 2005 and others entered the feedlot on November 2, 2005. Cattle were fed a receiving diet composed of 30% corn silage, 30% soybean hulls, 10% dry, whole shelled corn, and 30% supplement until the experiment began on November 22, 2005. The receiving diet contained 19% crude protein on a dry matter basis.

At the start of the trial, cattle were fed one of two finishing diets; one formulated with dry whole shelled corn (**DWSC**) as the main energy source and the other formulated with high moisture corn (**HMC**) as the main energy source. Compositions of the basal diets that were fed during the course of the trial are presented in Table 4.1; the diets were balanced on available energy source. The high moisture corn was fed whole at 76% of the diet on a dry matter basis. However, beginning the eighth week of the trial the 76% HMC in the HMC diet was fed 50% whole and 50% lightly rolled for approximately 12 weeks due to limitations in the storage capacity of the whole HMC. Cattle were restricted fed during the first two weeks of the trial to control feed intake and were then allowed ad libitum feed access for the remainder of the finishing period.

Cattle were fed on a pen basis and the cattle were randomly allotted to pens such that the average weight of each pen was equal. There were 24 pens used in the study (7 animals per pen). Each pen of steers was fed a basal diet that consisted of either DWSC or HMC (24.8% moisture). Each of the dietary energy sources was tested alone (Control; **CON**) or with the inclusion of Amaferm (**AMF**) or Levucell SB (**LEV**). Pen assignments for diet and treatment combinations were assigned randomly to the 24 pens. Feed additives (LEV or AMF) were mixed with dry, ground corn and then top dressed onto the remainder of the ration in the feed bunk. Those cattle receiving LEV received 0.5 g per head per day of the feed supplement. This dose was obtained by forming a pre-mix of 99.89% dry, ground corn and 0.11% LEV that was subsequently top dressed onto the respective diet of the pen. A pre-mix of 99.339% dry, ground corn and 0.661% AMF was top dressed onto pens receiving a diet supplemented with AMF to allow AMF to be supplemented at 3.0 g per head per day. Pens on the control diet, receiving no feed supplement, were fed dry, ground corn with no feed additive which was mixed with all other feed ingredients for that pen and fed as part of the total mixed ration. All steers were fed once daily at 0800.

Cattle were fed at the beef feedlot facility located at the Ohio Agricultural Research and Development Center in Wooster, Ohio. Pens were constructed of metal gates and cables, had slatted concrete floors, and were located in an open-sided barn. Pens were arranged in such a way that adjacent pens shared a common water source. All experimental procedures were approved by The Ohio State University Animal Care and Use Committee.

Performance Measurements

Initial and final weights were calculated as the average of weights obtained on two consecutive days at the beginning and end of the experiment. Cattle were assigned to 24 pens such that the average initial BW for cattle in each pen was equal. Cattle were weighed at 14-day intervals throughout the finishing period to monitor growth performance. Average daily gain (ADG), dry matter intake (DMI), feed efficiency (kg gain/kg feed), and days required to reach harvest weight were determined for all pens. The experiment was terminated when cattle were deemed by visual appraisal and body weight to be ready for harvest.

Carcass Data

Cattle were shipped and harvested at one of two locations; a commercial harvest facility or The Ohio State University Meat Science Abattoir. Due to limitations on slaughter capacity and constraints associated with sample collection, cattle were harvested over a six week period of time. Cattle were harvested on a pen basis and each harvest date contained an equal representation of experimental treatments. Carcass data, including those factors used to determine quality and yield grades, were collected at both harvest facilities by a USDA grader.

Bacterial cultures

On the second day of initial weighing, rectoanal swabs and fecal grabs were performed to determine the initial or baseline levels of *E. coli* O157:H7 and *Salmonella* spp. present prior to cattle being assigned to their respective treatments. Samples were

then taken at 14-day intervals throughout the finishing period during the time in which cattle were being weighed. Samples for testing *E. coli* O157:H7 were obtained by swabbing the rectoanal mucosa of each animal with sterilized dry cotton-tipped 15-cm cleaning sticks (Khaitsa et al., 2005). Immediately following swabbing, rectal fecal grabs from each individual animal were obtained by hand using disposable sleeves. The feces from the fecal grabs were then divided into two sterile tubes, one for determination of *E. coli* O157:H7 and the other for determination of *Salmonella.*

Salmonella sampling procedures:

Fecal grabs were collected from each animal at the initiation of the feeding period and concurrently while cattle were being weighed every two weeks throughout the finishing period. Fecal samples were transported to the Veterinary Lab in Columbus, OH where they were cultured for *Salmonella* using standard techniques described by Funk et al. (2000; 2001). Briefly, 4 g of feces was weighed at the laboratory and was incubated in 36 ml of Tetrathionate Broth (TTB) for 24 hours at 37°C. Following incubation, 100-µl of the TTB was inoculated into 10 ml of Rappaport-Vassiliadis broth (RV) and incubated for 24 hrs. at 42°C. Finally, an aliquot of RV was plated on XLT-4 agar and was incubated at 37°C for 24 hrs. Colonies exhibiting typical *Salmonella* morphology were further evaluated using biochemical tests. A positive control was subjected to the same isolation techniques as the fecal samples for every sampling period.

E. coli O157:H7 sampling procedures:

Each individual animal was tested for *E. coli* O157:H7 by swabbing the rectoanal mucosa with a sterilized dry cotton-tipped 15-cm cleaning stick (Khaitsa et al., 2005). Immediately after sampling, the swab was placed into a tube containing 3 ml of Buffered

Peptone Water (BPW). All tubes were transported to the Food Animal Health Research Program laboratory in Wooster, OH after sampling was complete. Rectoanal mucosal swab (RAMS) samples were incubated for 18 h at 42°C. Following incubation, AutoimmunoMagnetic Bead Separation (AIMS) (Dynal, Oslo, Norway) was performed on the RAMS samples and then plated onto Sorbital MacConkey agar plates (Difco Laboratories) containing cefiximine (50µl/ml) and potassium tellurite (2.5 mg/ml) ($SMAC_{CT}$). Plates were incubated at 37°C for 24 h. Using sterile toothpicks, up to 5 colonies from AIMS $SMAC_{CT}$ plates were transferred to 96 well plates containing 180-µl of *E. coli* 4-methylumbelliferyl-β-D-glucuronide (EC Mug) agar. On each plate, 1 well was inoculated as a positive control and 1 well was inoculated for a negative control. Plates were incubated at 37°C for 24 h. EC Mug fluorescence of 96 well plates was screened and colonies that fluoresced were transferred, using a 96 well replicator, to 150 x 15 mm MacConkey Agar (MAC) plates. MAC plates were incubated at 37°C for 18 h. MAC plates were screened for suspect colonies exhibiting purple colony morphology indicating lactose fermentation. Oxoid latex agglutination assay was used to confirm the O157 antigen.

In addition to RAMS, fecal grab samples were also cultured for *E. coli* O157:H7. Briefly, 10g of feces was homogenized in 90 ml of BPW to make a 10^{-1} dilution. Using 96 well plates containing 1 ml BPW, serial dilutions were made starting with the 10^{-1} dilution aliquot up to 10^{-3}. A 100-µl sample of each 10^{-3} dilution was spread plated onto 100 x 15 mm $SMAC_{CT}$ plates. Plates were incubated at 37°C for 18 hrs. $SMAC_{CT}$ plates were screened for colorless colony growth and plates were enumerated. Oxoid latex agglutination assay was used on suspect colonies to confirm the O157 antigen.

Statistical analyses

Data were analyzed as a 2 x 3 factorial with the main effects of dietary energy source (DWSC or HMC) and feed supplement (AMF, LEV, or CON). Pen was used as the experimental unit. Prevalence of *E. coli* O157:H7 or *Salmonella* spp. was defined as the proportion of cattle in a pen shedding a detectable level of *E. coli* O157:H7 or *Salmonella* spp. on any given sampling day. High shedders were defined as those animals that tested positive by direct plating onto $SMAC_{CT}$ plates, allowing for a count of *E. coli* O157:H7 to be obtained. Differences in growth performance, carcass characteristics, and prevalence of *E. coli* O157:H7 and *Salmonella* spp. between the different treatments were determined using the GLM function of SAS (Statistical Analysis System, Cary, N.C., 1999). The model included dietary energy source, feed supplement, and the interaction between energy source and feed supplement. The prevalence of *E. coli* O157:H7 between the two dietary energy sources was also analyzed by time using the GLM function of SAS. Means were separated using Fisher's Protected LSD test. Differences were considered significant after $P < 0.05$. Data were also examined to identify if particular cattle showed trends of consistently excreting high levels of *E. coli* O157:H7 and *Salmonella* using the PROC FREQ functions of SAS.

RESULTS AND DISCUSSION

Growth Performance

Results for those traits impacting feedlot performance are presented in Table 4.2. Main effects are presented because there was only one interaction observed between energy source (dry whole shelled corn, DWSC; high moisture corn, HMC) and

supplement type (control, CON; Levucell SB, LEV; Amaferm®, AMF). Initial and final body weights of steers were similar for all treatments (P > .05). During the finishing period, gains of steers did not differ between the different energy sources or supplement types (P > 0.05), although HMC tended (P = .09) to increase ADG in cattle compared to DWSC. Dry matter intake was similar among supplement types. However, a difference (P < .05) in DMI was observed between the two energy sources, such that those steers receiving the high-moisture corn diet consumed less feed on a daily dry matter basis than those steers fed dry, whole-shelled corn (8.18 kg vs. 8.38 kg). Ladely et al. (1995) reported that cattle fed HMC consumed less DM, gained faster, and were more efficient than cattle fed dry-rolled corn.

Results on feed efficiency indicated that an interaction occurred between energy source and supplement type (Fig. 4.1). AMF improved (P < 0.05) feed efficiency (.208 vs. .194 kg gain/kg feed) when DWSC was fed but not when HMC was fed. This improvement was primarily driven by a slight decrease in DMI in steers fed DWSC with AMF compared to steers fed DWSC with no supplement (8.20 kg vs. 8.60 kg), although this difference was not significant (P > .05). No previous studies have reported the effects of AMF on growth performance or feedlot characteristics, but Amaferm has been shown to increase populations of bacteria in vitro and within the rumen (Beharka et al., 1991; Beharka and Nagaraja, 1993; Varel and Kreikemeier, 1994). These microbes in the rumen, as well as in the cecum, ferment carbohydrates to volatile fatty acids and lactate (Owens et al., 1998). An increase in rumen bacteria would be expected to increase the amount of fermentation within the rumen, and hence the amount of fermentation end-products, which the animal uses for energy production. Amaferm has, in fact, been

reported to increase total volatile fatty acid concentrations, particularly concentrations of acetate and propionate, although others have found AMF to have no effect on VFA production (Nisbet and Martin, 1990; Gomez-Alarcon et al., 1990; Beharka et al., 1991). An increase in VFA production via stimulation of rumen bacteria may explain why an improvement in feed efficiency was seen when AMF was supplemented in the DWSC diet. An increase in VFAs would allow for more energy production by the animal and hence better utilization of feed. More energy production per unit of feed would allow for the animal to obtain its net energy requirements without consuming as much DM, thus leading to the improvement in feed efficiency that was observed.

Interestingly, there was no difference ($P > .05$) between AMF and CON fed cattle in the HMC diet. This most likely resulted because the magnitude of the difference in DMI between AMF and CON cattle fed DWSC was greater than the difference in DMI between AMF and CON cattle fed HMC. The effects of AMF have been demonstrated to be influenced by diet and forage type which may explain the different effects AMF had on the two corn types (Beharaka and Nagarja, 1993). However, most previous studies have looked at the effects of AMF with forage-based diets as opposed to concentrate-based diets.

Carcass Characteristics

The results for traits related to carcass merit are shown in Table 4.3. There were no interactions between energy source and supplement type observed; therefore the main effects are presented. Neither energy source, nor supplement type had a significant impact on any carcass traits measured in the study. In support of these findings, Huck et

al. (1998) reported no differences in carcass characteristics between different corn processing methods when fed in combination with steam-flaked grain sorghum. Because Levucell SB was designed specifically for monogastric application, it was expected to have no impact on carcass merit in the current study. This product was primarily used to determine its efficacy to reduce or eliminate pathogens such as *E. coli* O157:H7 and *Salmonella* spp. in the ruminant animal. There have been no previous studies that have looked at the impact of Amaferm on carcass characteristics in feedlot cattle. In a review by Krehbiel et al. (2003), it was reported that dressing percentage, quality grade, or percentage USDA choice of carcasses from feedlot steers were not influenced by direct-fed microbials containing varying concentrations and strains of *Lactobacillus acidophilus* and *Priopionbacterium freudenreichii.*

Prevalence of E coli O157:H7

Rectoanal mucosal swabs and fecal grab samples were obtained from each individual animal at 14-day intervals throughout the finishing period to detect for the presence of *E. coli* O157:H7. Prevalence was defined as the average percentage of positive cattle within a pen. A baseline sample was collected from each steer to assess the presence of *E. coli* O157:H7 prior to the start of the experiment. *E. coli* O157:H7 was isolated from 7 (4.2%) of the 168 steers during the baseline sampling which was conducted in conjunction with the initial weighing of the cattle. Only one of the animals was identified to be a excreting high levels of *E. coli* O157:H7 which was designated to an animal if it was found to be positive by direct plating onto $SMAC_{CT}$ plates. Three of the animals that were positive during the baseline sampling were subsequently assigned

to the CON treatment, where 1 received HMC and 2 received DWSC. Three were assigned to the LEV treatment and within this treatment 1 received HMC and 2 received DWSC. One steer was allotted to the DWSC diet that was supplemented with AMF. *E. coli* O157:H7 was not isolated from any of these same animals on the following sampling period after they had been on test for 14 days.

Overall, *E. coli* O157:H7 was isolated from 187 (10.26%) of 1822 RAMS samples taken during the finishing period. This prevalence is similar to that reported by LeJeune et al. (2004) in which 13% of fecal samples obtained from commercial feedlot cattle were positive for *E. coli* O157:H7. Several other studies have reported a much lower prevalence of approximately 1.0% in beef feedlot cattle (Hancock et al., 1997; Galland et al., 2001). However, the later studies were longitudinal studies in which fresh fecal pats were collected from several pens within several feedlots, whereas the former study and the current trial studied the prevalence of *E. coli* O157:H7 within the same set of cattle over time. Additionally, the detection method used in this study and the study by LeJeune et al. (2004) included immunomagnetic bead separation to concentrate *E. coli* O157:H7 from enriched cultures. This procedure was not performed in the studies in which a lower prevalence of *E. coli* O157:H7 was found. Therefore, the differences in prevalence may be due to differences in sensitivity of detection between the different methods.

Ninety-one (54.2%) cattle tested positive at least once during the feeding period, resulting in 77 (45.8%) cattle which never tested positive for *E. coli* O157:H7. There were a few cattle that tested positive by direct plating onto $SMAC_{CT}$ plates, but were not found to be positive from a RAMS sample collected during the same sampling date. In a

similar study by Khaitsa et al. (2003) that looked at the prevalence, incidence, and duration of fecal shedding by a group of feedlot cattle over time, *E. coli* O157:H7 was recovered from each of the 100 steers at least once during the 19 week feeding period. In the current study, 58 (34.5%) cattle shed high amounts of the microorganism at some point during the finishing period as determined by direct plate counts. Approximately one-third of the cattle that produced a positive rectoanal swab sample were shedding *E. coli* O157:H7 at a level that resulted in a direct plate count (39.6%).

Peak prevalence of *E. coli* O157:H7 across all treatments and diets occurred on January 3, 2006, approximately 6 weeks after the commencement of the trial at which time the average prevalence of cattle shedding within a pen was 22%. Several studies indicate that the prevalence of *E. coli* O157:H7 is higher early in the feeding period, shortly after the cattle have entered the feedlot. This is attributed to the fact that this is a time in which the animals encounter many stresses, including adaptation to a different environment and diet, which can make them susceptible to infection with pathogenic microorganisms. Hancock et al. (1997) reported that within a feedlot, pens with cattle that had been on feed the shortest amount of time showed a threefold increase in the prevalence of *E. coli* O157. LeJeune et al. (2004) also reported that prevalence of *E. coli* O157 peaked 2 weeks after cattle entered the feed yard. In the current study, a portion of the cattle entered the feedlot on October 5, 2005 and others entered the feedlot on November 2, 2005. Thus, sampling did not begin on the first set of steers until approximately 7 weeks after they entered the feedlot. Therefore, the peak in fecal excretion of these animals may have occurred earlier on in the feeding period during the time in which sampling was not taking place. However, sampling on the second set of

steers began approximately 3 weeks after entering the feedlot, at which time prevalence was low for all cattle. Regardless, there was a peak 6 weeks after the beginning of the trial, after cattle had been weighed, resorted into pens, and placed on full feed. Therefore, it seems that in this study the peak in fecal excretion of *E. coli* O157:H7 was delayed in comparison to when the peak was seen in previous studies, or it was triggered by some other source of stress. In study by Khaitsa et al. (2003), it was reported that the incidence of *E. coli* O157:H7 did not show a dramatic increase until week 9 of the finishing period and reached the highest incidence in week 12. This pattern of excretion seems to possibly mimic that of the current study in which the peak in fecal excretion occurred 13 weeks and 9 weeks after the first and second group of cattle entered the feedlot.

Weather statistics for each of the 10 sampling periods in which all cattle were tested are presented in Table 4.4. Each statistic represents the average for the 14 days prior to the date in which samples were obtained. Weather has been reported to affect the excretion of *E. coli* O157:H7 in ruminant animals causing a higher incidence of positive animals in warmer and moister conditions (Kudva et al., 1996). The 14 days prior to the peak in excretion were characterized by a greater increase in temperature compared to the 14 days prior to the previous sampling date. The average temperature for this period of time was 8.6°C higher than the average temperature of the previous sampling period (2.9°C vs. -5.7°C). The average high temperature was 7.4°C higher and the average low temperature was 10.6°C higher than the averages for the 14 days prior to the previous sampling date. Additionally, the day prior to sampling on January 3, 2006 there was nearly seven-tenths of precipitation. Therefore, the spike in fecal excretion of *E. coli*

O157:H7 that occurred 6 weeks after the beginning of the trial may have been weather-related.

Effect of feed additive

The pattern of *E. coli* O157:H7 excretion was similar between the three treatments, AMF, LEV, and CON, throughout the trial (Fig. 4.2). Thus, inclusion of AMF or LEV in the diet had no significant impact on the prevalence of *E. coli* O157:H7 in feces throughout the entire feeding period. The peak in the percent of positive animals within a pen for those pens receiving the AMF treatment was not as great as the peak seen in those pens receiving the CON or LEV treatments, although this difference was not significant ($P > 0.05$). The pattern for the average percent of high shedders within a pen was also similar for all three treatments throughout the feeding trial (Fig. 4.3).

AMF has been shown to increase endogenous populations of ruminal bacteria and increase the growth rate and activity of rumen fungi (Beharka and Nagaraja, 1998; Chang et al., 1999). It was hypothesized that through this increase in competing beneficial bacteria within the digestive tract, addition of Amaferm to the ruminant diet may reduce the presence and proliferation of pathogenic microorganism within the animal. Additionally, it was suggested that due to the increase in fermentable fiber from the increase in rumen fungi, there would be a subsequent increases in fermentation end products such as short-chain fatty acids. An increase in short chain fatty acids would lead to a decrease in the pH of the colon and possibly create an unfavorable environment for the survival of pathogenic bacteria. However, in this study AMF was not found to be efficacious in reducing the overall prevalence of *E. coli* O157:H7 in feedlot cattle.

LEV contains the CNCM I-1079 strain of *S. cerevisiae*, ssp. *boulardii* which has been reported to have the ability to bind to enteric pathogens, including *E. coli* O157:H7, preventing adhesion of this pathogen to the mucosal membrane of the gut which is crucial to the virulence of this microorganism (Gedek, 1999). In this study LEV did not appear to influence the presence and proliferation of *E. coli* O157:H7 within the gastrointestinal tract, as excretion of *E. coli* O157:H7 in feces of cattle receiving LEV was comparable to that of CON cattle. However, in the current study LEV was fed to provide only 0.5 g per head per day, which may not have been a high enough dose to cause an observable response. Further work is needed to determine if LEV may be efficacious in reducing pathogen prevalence when fed at higher doses.

Effect of dietary energy source

As shown in Figure 4.4, dietary energy source appeared to impact the fecal excretion of *E. coli* O157:H7 among feedlot steers. The average percentage of positive animals within a pen was similar among the two corn diets during the first 6 weeks of the experiment. However, after the peak in excretion, which occurred on January 3, 2006, the pattern of excretion began to differ between cattle receiving the two different diets. The percent of animals within a pen excreting *E. coli* O157:H7 for pens receiving HMC declined to levels near those seen prior to the peak. The average percent of positive cattle within a pen for those pens on the DWSC diet was greater for several weeks after the peak in fecal excretion occurred. In fact, a significant decline in the prevalence of *E. coli* O157:H7 among pens receiving DWSC was not seen until approximately 8 weeks after the peak in excretion occurred. Thus, it appears that cattle fed HMC were more resilient

to the sudden increase in *E. coli* O157:H7 excretion observed on the January 3rd sampling date. Similar results were seen when analyzing the prevalence of only high shedders within a pen (Fig. 4.5). The percent of high shedders in a pen receiving HMC peaked on January 3, 2006 and then declined shortly thereafter to levels seen prior to the peak. However, the percent of high shedders in pens receiving DWSC remained greater until approximately 8 weeks after the peak occurred.

Although several researchers have looked at the impact of diet on the fecal excretion of *E. coli* O157:H7 in cattle, no studies have looked at the effect of HMC vs. DWSC on the prevalence of this pathogen. Many studies report the effects of grain-based vs. forage-based diets on *E. coli* O157:H7 excretion, but no consistent results have been found. Hovde et al. (1999) and Kudva et al. (1997) both found grass or hay-fed animals to excrete *E. coli* O157:H7 for a longer duration and in larger numbers than grain-fed animals, however, others have reported no difference between the two (Tkalcic et al., 2000). Abruptly switching cattle from grain to forage just prior to slaughter may potentially reduce *E. coli* O157:H7 populations in cattle (Callaway et al., 2003b). Among grain-fed cattle, the feeding of barley has been reported to increase the likelihood of a pen of cattle within a feedlot being positive for *E. coli* O157 compared to cattle fed corn or other types of grains (Dargatz et al., 1997; Buchko et al., 2000a; Berg et al., 2004).

The current study made use of HMC and DWSC. HMC is thought to ferment much more rapidly than DWSC within the rumen because of the availability of the starch granules. As corn dries, starch becomes more compact, making it less available for fermentation by rumen microorganisms. The increase in fermentation rate of the HMC

may have caused an increase in volatile fatty acids within the rumen, lowering the pH and creating an unfavorable environment for *E. coli* O157:H7. This may be a reason as to why steers fed HMC exhibited a lower prevalence of *E. coli* O157:H7 excretion in feces compared to steers fed DWSC. However, it has been shown that *E. coli* O157:H7 primarily colonizes the hindgut or colon, which is where a greater proportion of the DWSC would have most likely been fermented due to its slow rate of fermentation causing it to escape fermentation in the rumen. Therefore, it would be expected that DWSC would have been more capable of lowering the pH in the area of the gastrointestinal tract that is thought to harbor the greatest population of pathogenic microorganisms. The exact reason for the reduction in excretion of *E. coli* O157:H7 that was seen in HMC-fed steers compared to steers fed DWSC warrants further investigation.

Incidence and Duration of E. coli O157:H7 excretion

Over time, the percentage of pens with at least one positive RAMS sample was similar, regardless of feed additive (Fig. 4.6). Additionally, the incidence of a pen having at least one high shedder was similar between diets containing feed additives and the control diet throughout the finishing period; thus, further supporting the previous conclusions that in this study, these additives had no effect on reducing the excretion of *E. coli* O157:H7 during the finishing stage.

The effect of dietary energy source on the persistence of *E. coli* O157:H7 excretion can be seen in Figure 4.7. The distributions shown in Figure 4.7 indicate that pens receiving DWSC had a greater number of pens with at least one positive RAMS

sample for a greater number of weeks than pens receiving HMC. In fact, one pen on the DWSC diet had at least one positive RAMS sample for 9 out of the 10 weeks in which all pens were sampled. A similar skew in the distribution between pens of cattle receiving DWSC and HMC was seen for the number of weeks a pen had at least 2 or more and 3 or more positive RAMS samples. One pen of cattle that received the DWSC diet, and two pens of cattle that received the HMC diet did not produce any positive *E.* coli O157:H7 samples during the 10 weeks of sampling. However, 10 of the 12 pens of cattle that received the DWSC diet had at least two cattle produce positive *E.* coli O157:H7 samples during the 10 weeks of sampling versus only six of the 12 pens of cattle receiving the HMC diet. The same trend was seen in pens that contained at least three positive *E.* coli O157:H7 samples during the 10 weeks of sampling with seven of the 12 pens versus four of the twelve pens cattle producing positive *E. coli* O157:H7 samples for the DWSC and HMC diets, respectively. Additionally, a greater percentage of pens on DWSC had at least one positive RAMS sample on sampling dates after the spike in *E. coli* O157:H7 fecal excretion occurred compared to pens on HMC (Fig. 4.8). A similar trend was seen for the percentage of pens with 2 or more and 3 or more positive RAMS samples. There was also a greater incidence in the percentage of pens with at least one high shedder in pens receiving DWSC than in pens receiving HMC after the spike in shedding (Fig. 4.9). This may offer an explanation as to why the percentage of positive animals within a pen remained high for such a long time after the peak for pens receiving DWSC, whereas a decline in the percentage of positive animals within a pen was seen in pens receiving HMC. Thus, it appears that collectively animals in pens receiving DWSC excrete *E. coli*

O157:H7 in their feces for a longer duration and at a greater level than those animals in pens receiving HMC.

The frequency of positive animals on an individual basis between DWSC and HMC differed, as shown in Figure 4.10. The percent of cattle that tested positive multiple times was greater for animals fed DWSC than for animals fed HMC. One animal fed the DWSC diet cultured positive 7 out of the 10 sample periods in which all pens were tested. It did not appear as if this animal caused infection or excretion in other animals in the pen or in animals in adjacent pens. The percent of animals in which *E. coli* O157:H7 was never detected was significantly greater for cattle fed HMC than for cattle fed DWSC (58.3% vs. 33.3%). Those steers fed DWSC were 25% more likely to be excreting *E. coli* O157:H7 than steers fed HMC and were 24% more likely to be excreting high amounts of *E. coli* O157:H7 than steers fed HMC (Fig. 4.11). Again, it appears that cattle fed HMC were more resilient and less susceptible to infection with *E. coli* O157:H7 than cattle fed DWSC. No animal was ever determined to be a high shedder more than 3 times during the finishing period and thus there were no animals identified as being the primary culprits for increasing or continuing the incidence of shedding with a pen of cattle.

Prevalence of Salmonella

Salmonella was isolated from none of the fecal samples collected from the 168 steers during the initial weighing period prior to the start of the experiment. Thus, *Salmonella* was not initially present in any animal. Cattle were sampled every 14 days during the finishing period, beginning December 4, 2005 until the last of the cattle were

harvested on May 8, 2006. This resulted in a total of 11 sampling periods. A fecal sample was obtained from every animal in each of the 24 pens during each sampling period except for the last 2 sampling days in which samples were collected from only 20 pens and 8 pens, respectively, because the other pens of steers had already been harvested. No fecal samples from any animal tested positive for *Salmonella* during the entire finishing period. Consequently, statistical analysis to determine the effects of energy source and AMF or LEV supplementation on fecal excretion of *Salmonella* spp. was not performed. Other researchers have isolated *Salmonella* from feces of feedlot cattle, although, frequency has been low (3 to 7%) (Dargatz et al., 2003; Beach et al., 2002). Similar results have been shown in cattle after transport at the harvest facility (2 to 7%) (Fegan et al., 2004; McEvoy et al., 2003). However, Fegan et al. (2005) reported the prevalence of *Salmonella enterica* in cattle during processing to be 16% in feces collected after evisceration. In the current study, cattle were housed in pens with slatted floors, which differs from the dry-lot pens that are typically found in feedlots. Because the pens had slatted floors that allowed fecal material to drop down into a pit below the pen, steers had limited contact with feces. Cattle often become infected with food-borne pathogens such as *Salmonella* from contact with infected feces of other animals within a pen. Persistence may occur once one animal in a pen becomes infected as a cycle may begin in which animals shed the organism in feces and become re-infected by exposure to their own or another animals infected feces. Because of the limited exposure to feces in cattle in this study, transmission and persistence of *Salmonella* among pen mates may have been prevented. Moreover, even if an animal in a pen shed *Salmonella* for a short time period, the amount being shed may have been below the detection limit of the

method used to isolate the microorganism. During the study, feces from cattle that were positive for *E. coli* O157:H7 were inoculated with a *Salmonella* positive control to determine if there was something present in the feces that may have been inhibiting the growth of *Salmonella,* however, there was no indication from these samples that an inhibitory agent was present.

IMPLICATIONS

Neither of the feed additives, AMF or LEV, were found to be effective in reducing the excretion of *E. coli* O157:H7 in cattle being finished on high concentrate corn-based diets. However, this study did provide evidence that cattle being finished on a high concentrate diet that utilizes high-moisture corn may be more resilient and may excrete *E. coli* O157:H7 at a reduced rate than those cattle being finished on a high concentrate diet formulated with DWSC. Further research is warranted to determine if HMC actually decreases the susceptibility to infection or colonization of *E. coli* O157:H7 in the gastrointestinal tract.

Item	Diet[a]	
	High-moisture Corn	Dry, Whole Shelled Corn
Ingredient	% DM basis	
Corn, high-moisture	76.000	—
Corn, whole shelled	—	76.000
Corn Silage	10.000	10.000
Soybean oil	3.000	3.000
Soybean meal 44%	7.415	7.415
Urea	1.050	1.050
Limestone	1.415	1.415
Trace Mineral Salt[b]	.480	.480
Vitamin A, 30,000 IU/kg	.005	.005
Vitamin D, 3,000 IU/kg	.009	.009
Vitamin E, 44,000 IU/kg	.009	.009
Selenium, 201 mg/kg	.048	.048
Rumensin-80[c]	.017	.017
Tylan-10	.048	.048
Potassium chloride	.394	.394
Animal-Vegetable Fat	.110	.110
Nutrient Composition		
Dry Matter, %	65.500	79.500
Organic Matter[d], %	96.900	96.900
Crude Protein[d], %	13.050	13.300
NDF[d], %	12.400	12.400
Calcium[d], %	.552	.552
Phosphorus[d], %	.311	.311
Potassium[d], %	.706	.706
Ne_m[d], Mcal/kg	2.234	2.234
Ne_g[d], Mcal/kg	1.547	1.547

[a] Diet was mixed with dry, ground corn or top dressed with dry, ground corn plus Amaferm (3 g/head/d) or Levucell SB (.5 g/head/day).

[b]Contained > 93% NaCl, .35% Zn, .28% Mn, .175% Fe, 0.035% Cu, .007% I, and .007% Co.

[c] Monensin, Elanco, Greenfield IN.

[d]Calculated using NRC (1996) values.

Table 4.1: Ingredient and nutrient composition of diets.

	Energy Source[a]				Supplement Type[b]				
	DWSC	HMC	P-value	SEM	CON	LEV	AMF	P-value	SEM
No. of pens	12	12	—	—	8	8	8	—	—
Initial weight, kg	299.7	300.0	.77	.76	299.9	299.2	300.4	.65	.93
Final weight, kg	551.4	558.3	.30	4.6	553.9	554.3	556.4	.94	5.6
Days on feed	150	150	1.00	3.9	149	150	150	.97	4.8
Gain, kg/d	1.69	1.73	.09	.02	1.71	1.71	1.71	.99	.02
DM intake, kg/d	8.38	8.18	.03	.06	8.36	8.29	8.19	.30	.08
Gain/feed, kg/kg	.202	.212	.002	.002	.205	.206	.209	.58	.002

[a] DWSC = dry, whole shelled corn; HMC = high-moisture corn

[b] CON = control; LEV = Levucell SB (.5 g/head/d); AMF = Amaferm (3 g/head/d)

Table 4.2: Mean values for growth performance traits by energy source and supplement type.

	Energy Source[a]				Supplement Type[b]				
	DWSC	HMC	P-value	SEM	CON	LEV	AMF	P-value	SEM
No. of pens	12	12	—	—	8	8	8	—	—
Hot carcass weight, kg	340	345	.33	3.4	342	343	343	.99	4.1
Dressing percent	61.7	61.8	.67	.18	61.8	61.9	61.6	.63	.21
Backfat, cm	1.32	1.40	.24	.05	1.30	1.36	1.42	.34	.06
Longissimus muscle area, cm^2	79.4	81.7	.13	1.1	79.9	80.7	81.1	.79	1.3
Kidney, pelvic, heart fat, %	2.3	2.4	.54	.16	2.3	2.5	2.327	.67	.2
USDA marbling score[c]	534	547	.30	9.2	551	539	532	.50	11.3
USDA quality grade[d]	4.94	5.12	.19	.09	5.13	5.01	4.95	.56	.12
USDA yield grade	3.17	3.20	.82	.09	3.14	3.21	3.21	.87	.11

[a] DWSC = dry, whole shelled corn; HMC = high-moisture corn

[b] CON = control; LEV = Levucell SB (.5 g/head/d); AMF = Amaferm (3 g/head/d)

[c] Marbling based on numeric scale of 500-590 = small

[d] Quality grade based on numeric scale in which 5 = low choice

Table 4.3: Effects of energy source and supplement type on carcass characteristics.

Sampling Date	Weather Statistics						% of steers positive for *E. coli* O157:H7	
	Average Temp[a], °C	Average High Temp[a], °C	Average Low Temp[a], °C	Maximum Temp[b], °C	Minimum Temp[b], °C	Precipitation[c], cm	RAMS[d]	Direct plating[e]
Nov. 21	6.89	12.72	0.78	21.67	-8.33	0.94	4%	1%
Dec. 6	-0.5	3.67	-4.5	19.94	-13.17	1.04	4%	1%
Dec. 20	-5.72	-1.28	-10	2.94	-17.06	0.64	8%	6%
Jan. 3	2.89	6.11	0.61	12.22	-6.22	1.75	22%	11%
Jan. 17	3.17	7.56	-0.11	16.5	-6.39	0.36	13%	5%
Jan. 31	3	7.94	-1.56	14.5	-7.67	0.84	11%	4%
Feb. 14	-0.94	2.5	-4.61	12.61	-10.67	2.03	11%	4%
Feb. 28	-0.67	5.39	-5.67	16.56	-15.11	0.36	7%	4%
Mar. 14	4.28	9.06	-0.72	20.39	-8.5	2.24	7%	2%
Mar. 28	0.89	5.39	-3.72	12.72	-7.17	0.23	6%	1%

[a]Temperature represents the average of the 14 days prior to the sampling date.

[b]Temperature represents the maximum reached on a single day during the 14 days prior to the sampling date.

[c]Amount of precipitation is the most obtained on a single day during the 14 days prior to the sampling date.

[d]Rectoanal mucosal swabs used to detect *E. coli* O157:H7 .

[e]Direct plating onto $SMAC_{CT}$ plates used to enumerate colonies and those animals with positive counts were determined to be high shedders of *E. coli* O157:H7.

Table 4.4: Effect of weather on the percent of culture positive RAMS samples and the percent of animals excreting high amounts of *E. coli* O157:H7 within each sampling period.

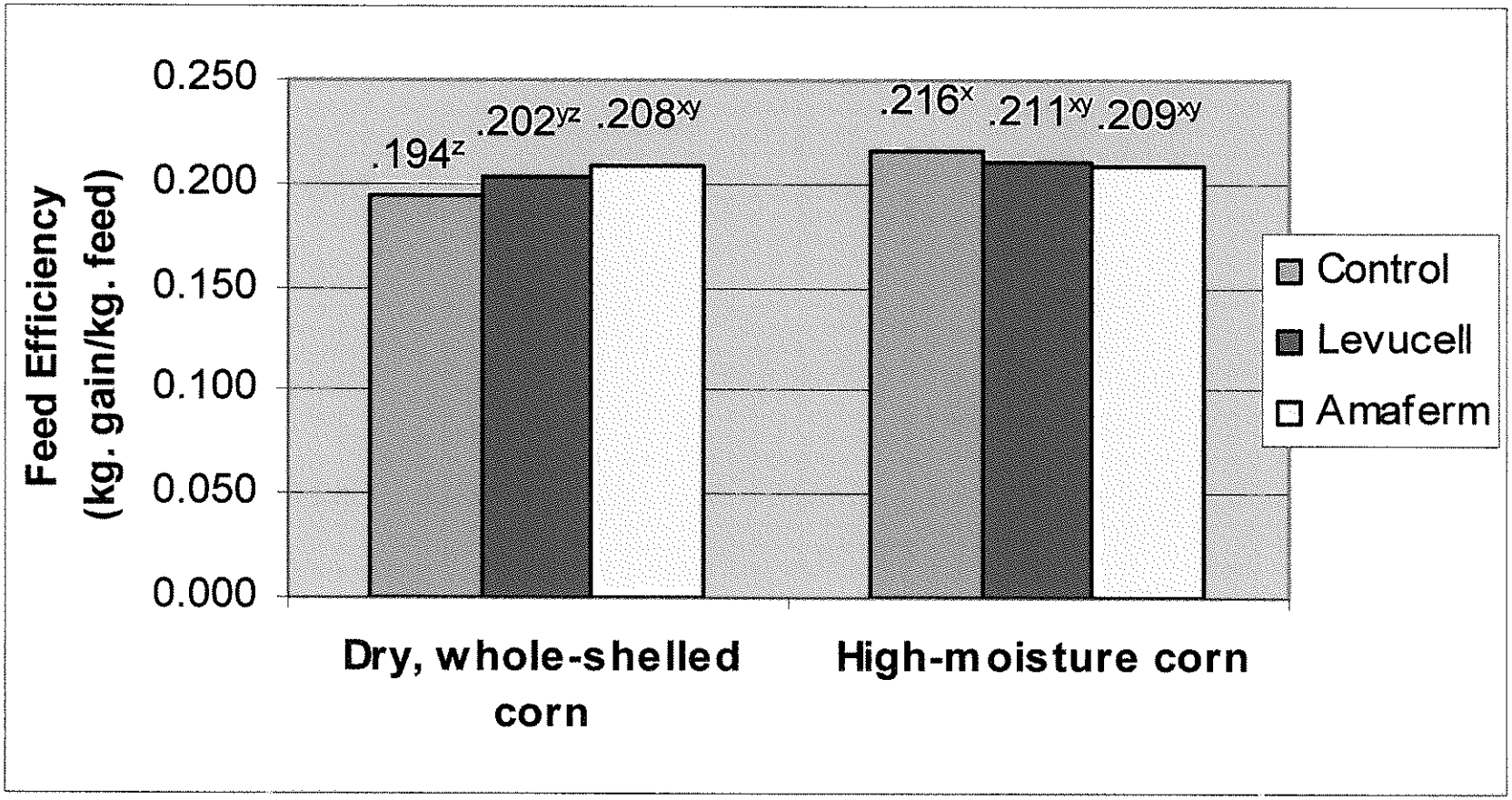


Figure 4.1: Interaction of energy source and supplement type on feed efficiency. (Values without a common letter in their superscript differ ($P < .05$).

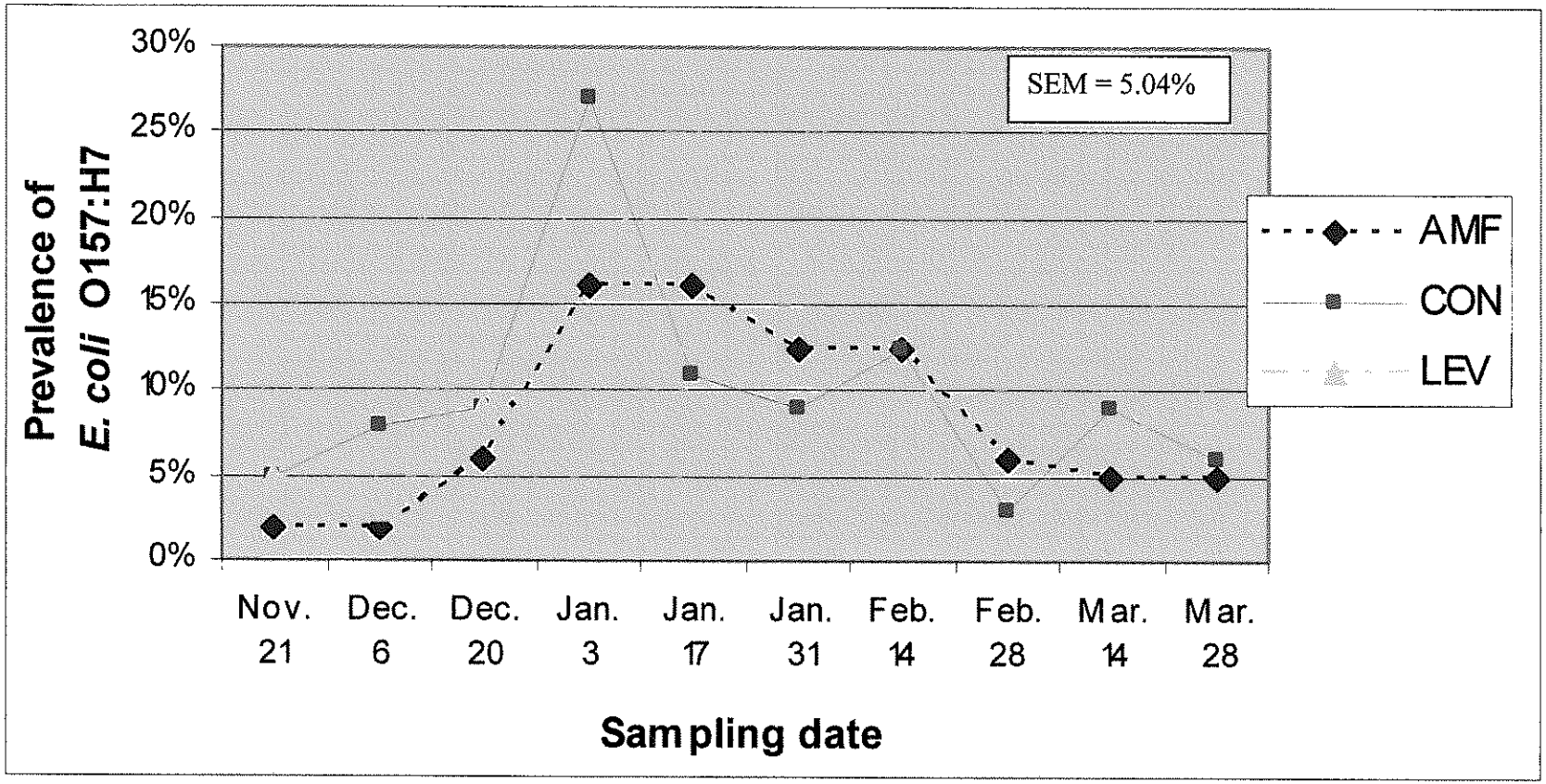


Figure 4.2: Effect of feed additive, Amaferm ® (AMF), Levucell (LEV) or no addative (Control; CON) on the average percentage of steers positive for *E. coli* O157:H7 within a pen at each sampling period as determined by rectoanal mucosal swabbing.

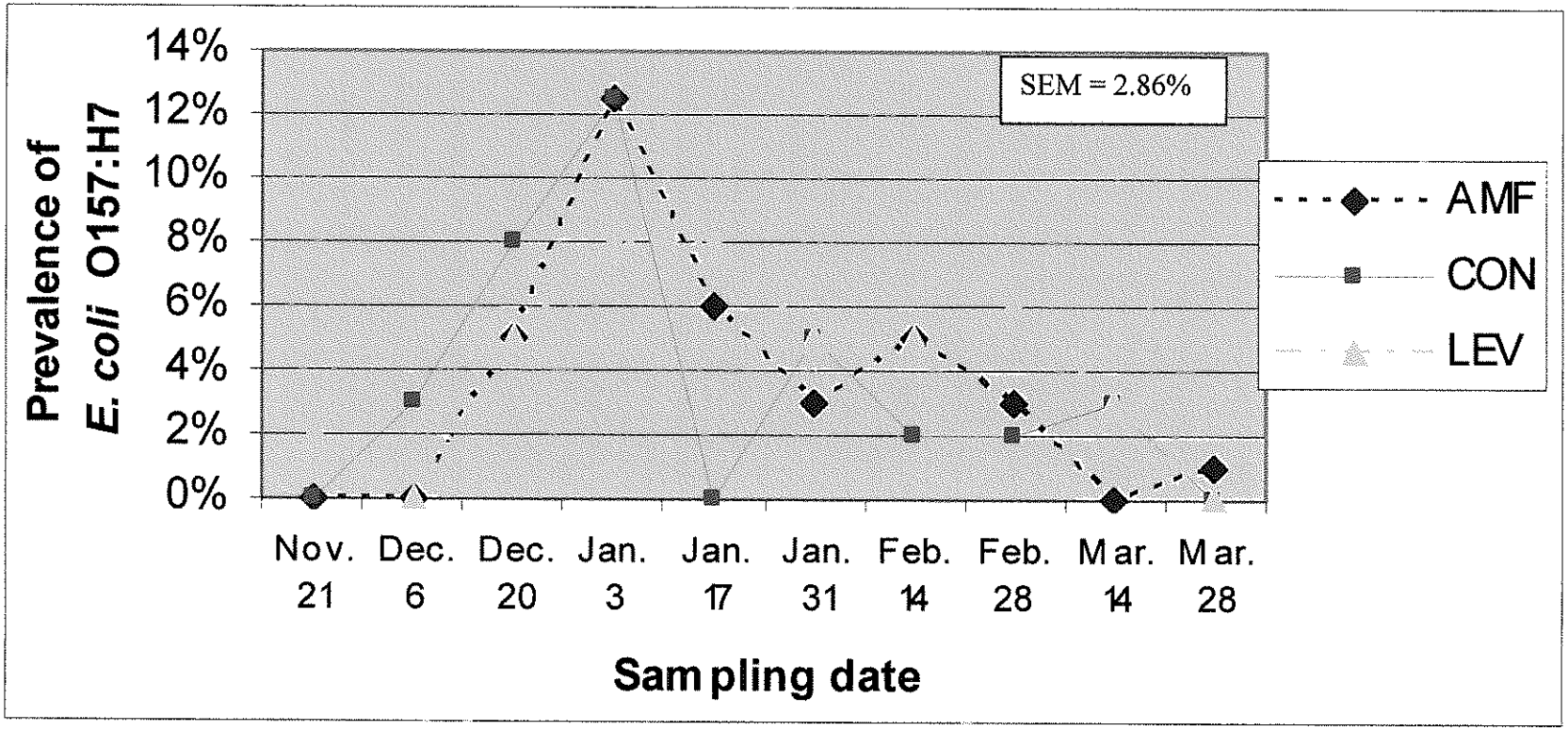


Figure 4.3: Effect of feed additive, Amaferm ® (AMF), Levucell (LEV) or no additive (Control; CON) on the average percent of high shedders of *E. coli* O157:H7 within a pen at each sampling period.

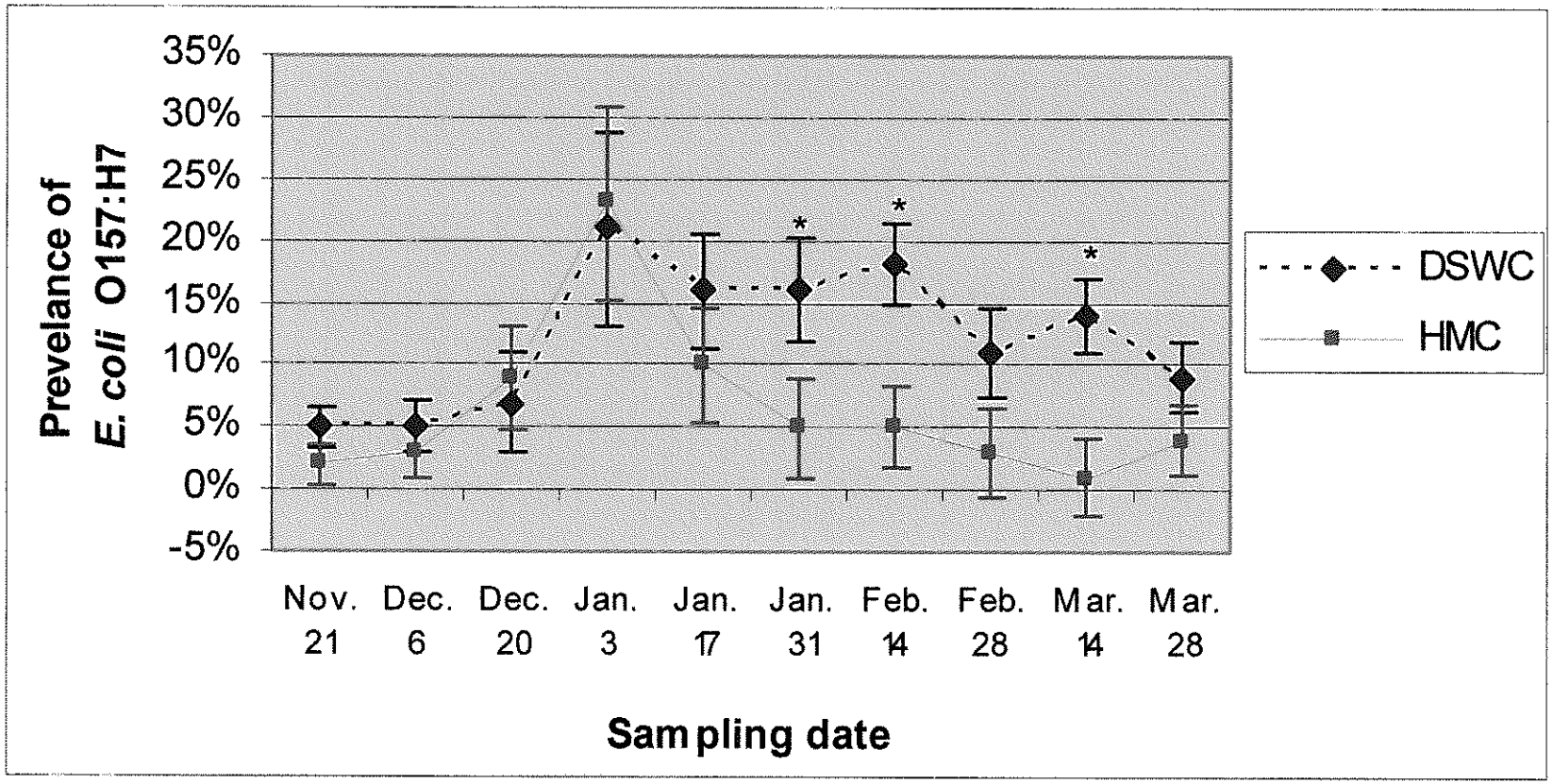


Figure 4.4: Effect of dietary energy source, dried whole-shelled corn (DSWC) or high-moisture corn (HMC) on the average percent of steers positive for *E. coli* O157:H7 within a pen at each sampling period as determined by rectoanal mucosal swabbing. (* signifies difference ($P < 0.05$) was observed within a time period)

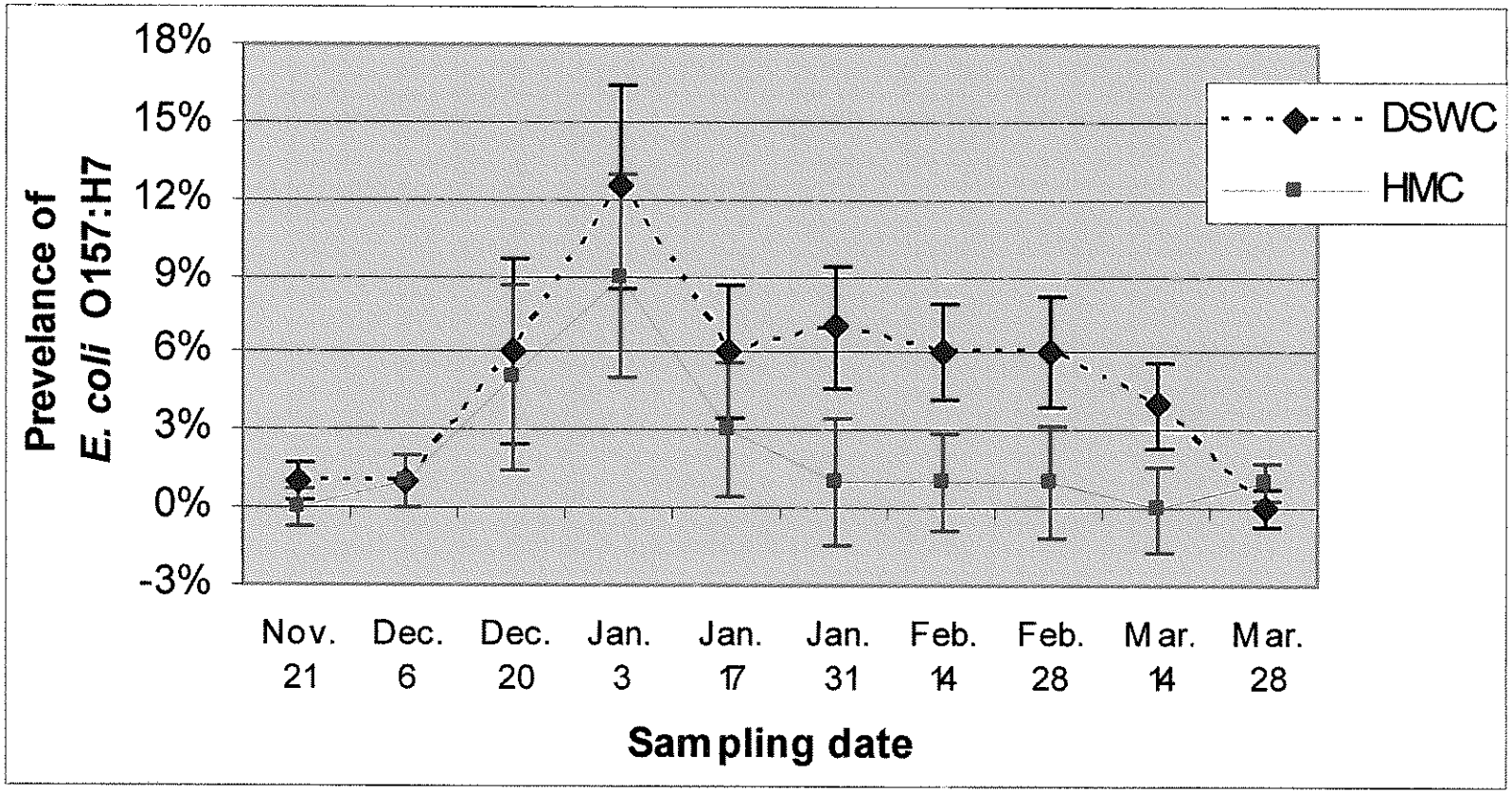


Figure 4.5: Effect of dietary energy source, dried whole-shelled corn (DSWC) or high-moisture corn (HMC), on the average percent of high shedders of *E. coli* O157:H7 within a pen at each sampling period.

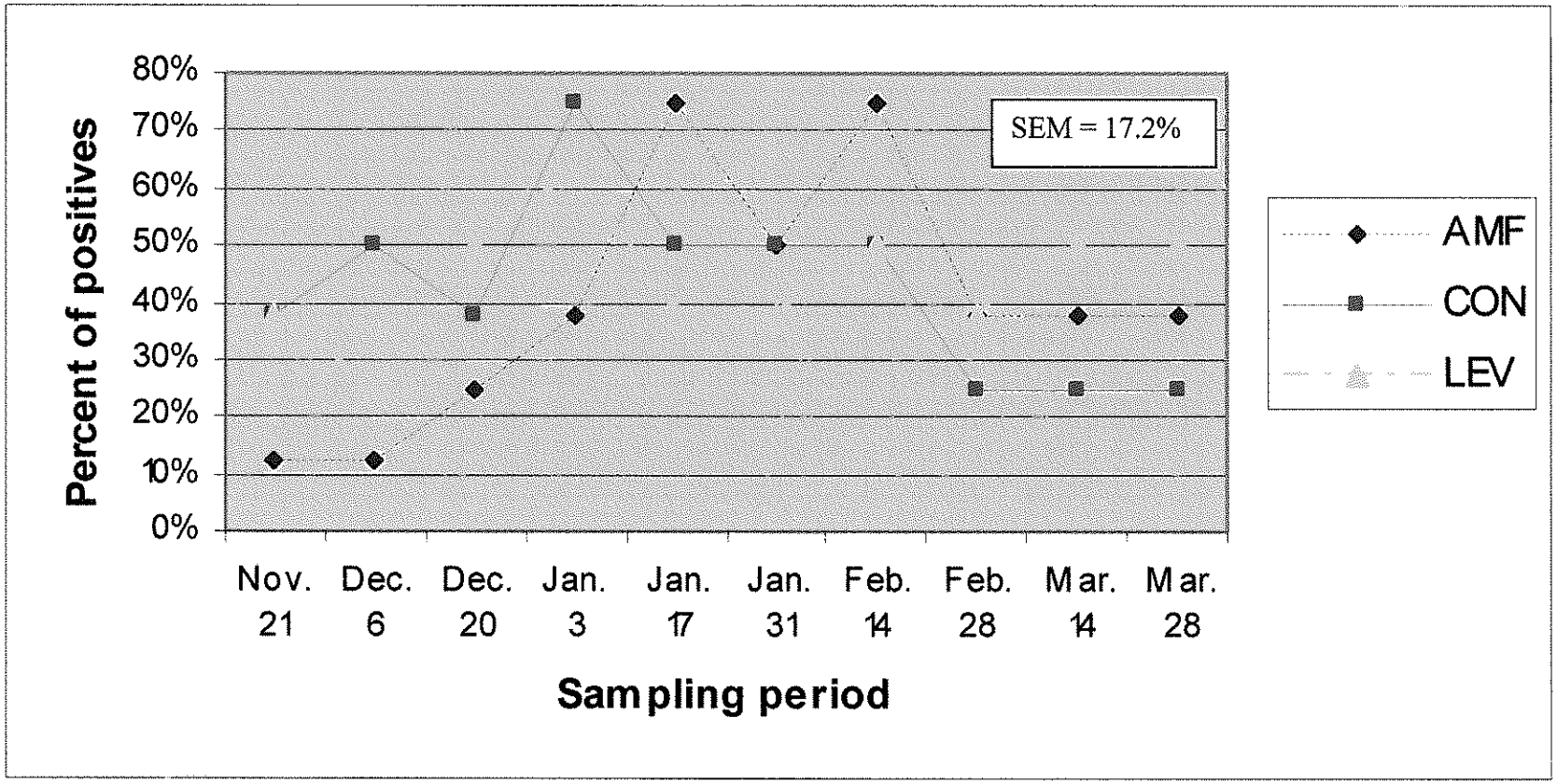
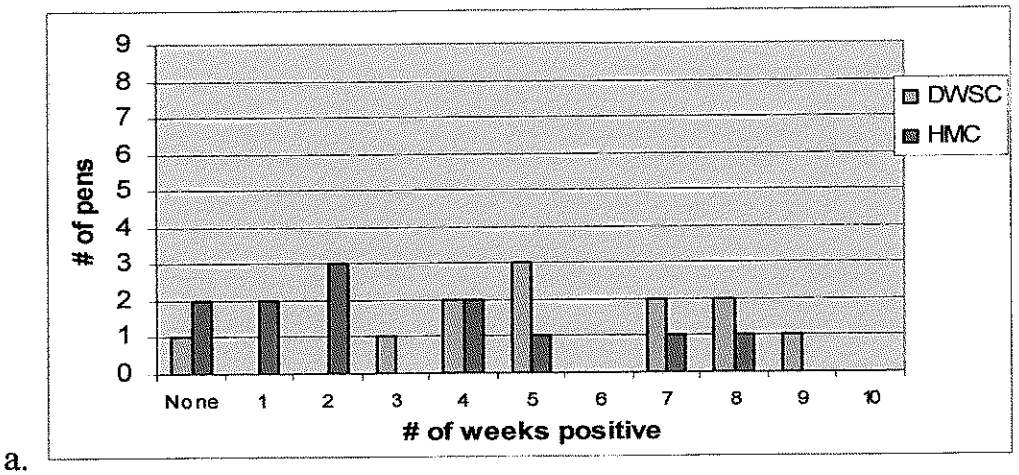
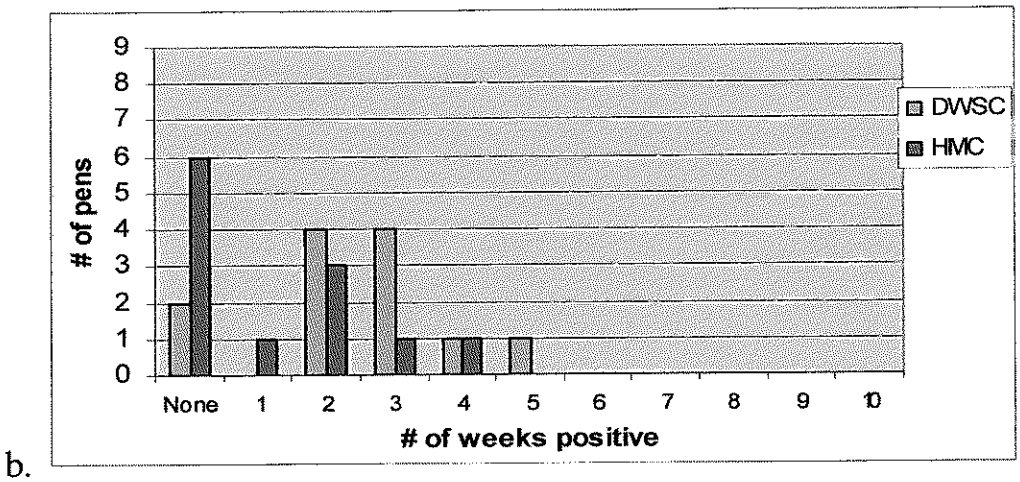


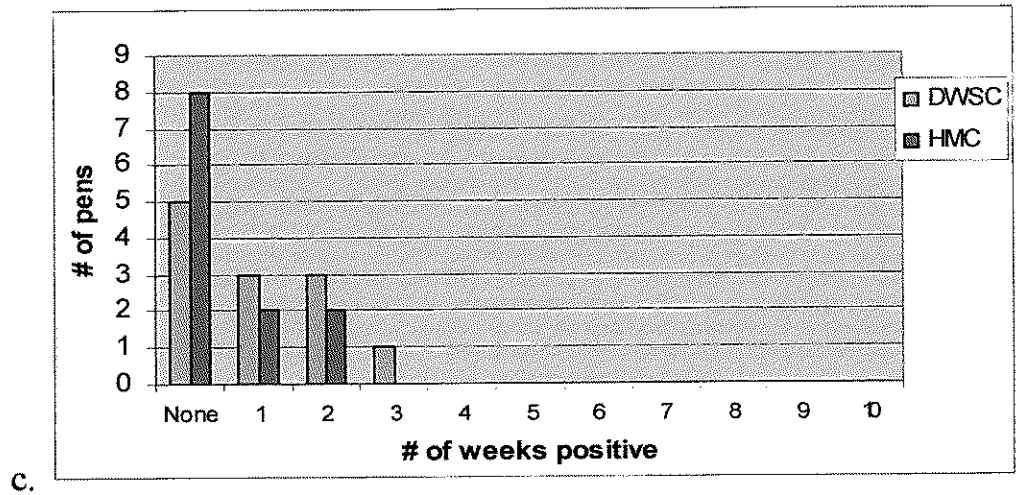
Figure 4.6: Percentage of pens on each supplement treatment, Amaferm ® (AMF), Levucell (LEV) or no addative (Control; CON), containing at least one animal that was culture positive for *E. coli* O157:H7 at each sampling period as determined by rectoanal mucosal swabbing.



a.

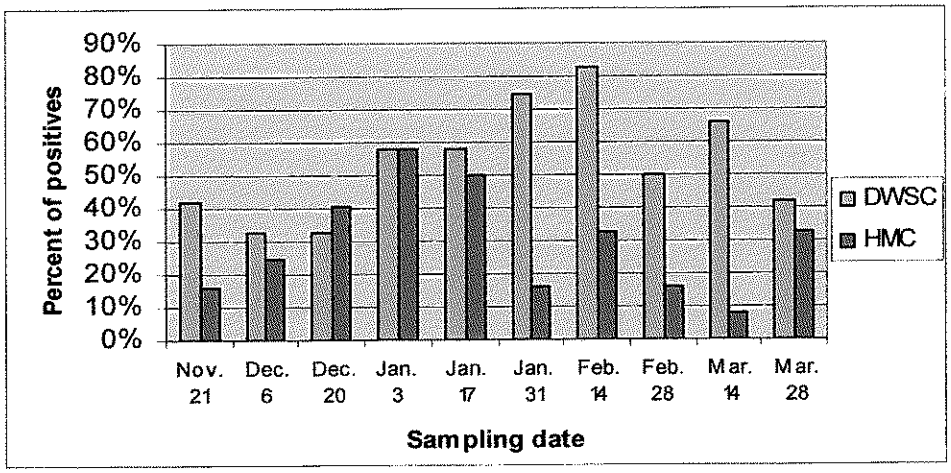


b.

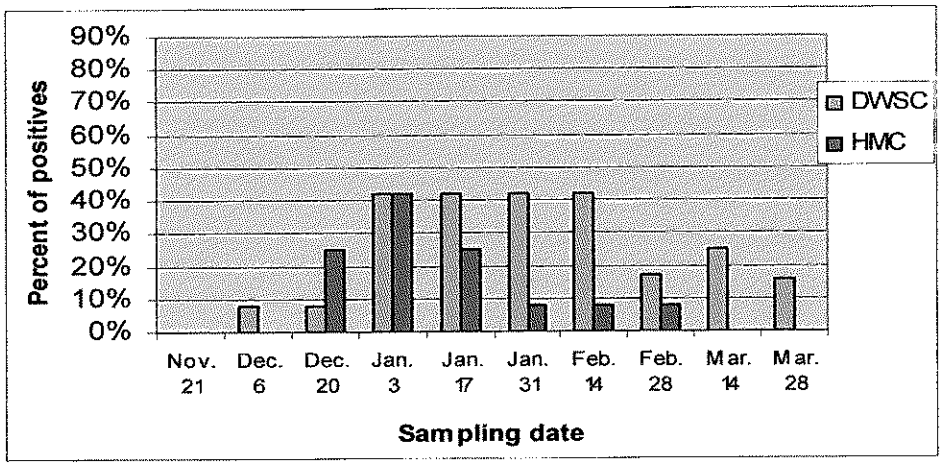


c.

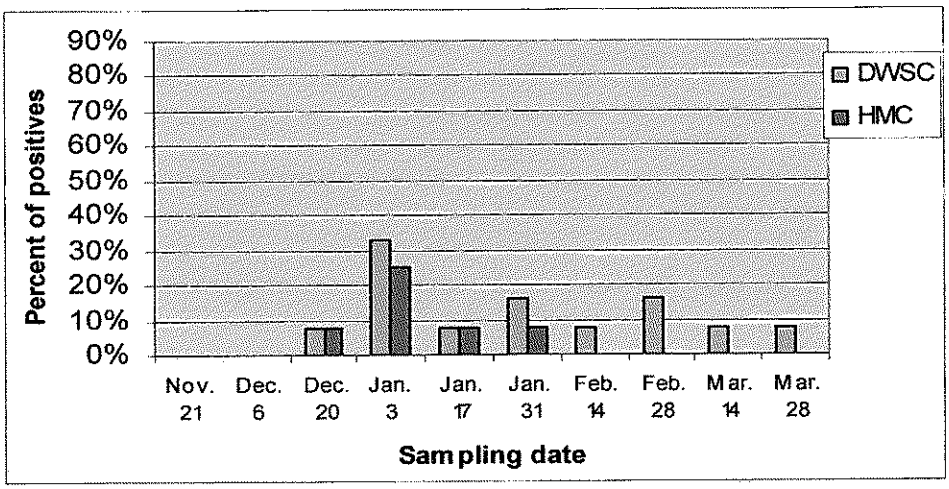
Figure 4.7: Distribution of the effect of energy source, dried whole-shelled corn (DSWC) or high-moisture corn (HMC), on the number of weeks a pen contained animals that cultured positive for *E. coli* O157:H7 as determined by rectoanal mucosal swabbing, a) pens that contained at least one positive animal, b) pens that contained at least two animals, c) pens that contained at least three animals.



a.



b.



c.

Figure 4.8: Effect of energy source, dried whole-shelled corn (DSWC) or high-moisture corn (HMC), on the percentage of pens containing animals that tested positive for *E. coli* O157:H7 as determined by rectoanal mucosal swabbing by sampling date, a) pens that contained at least one positive animal, b) pens that contained at least two animals, c) pens that contained at least three animals.

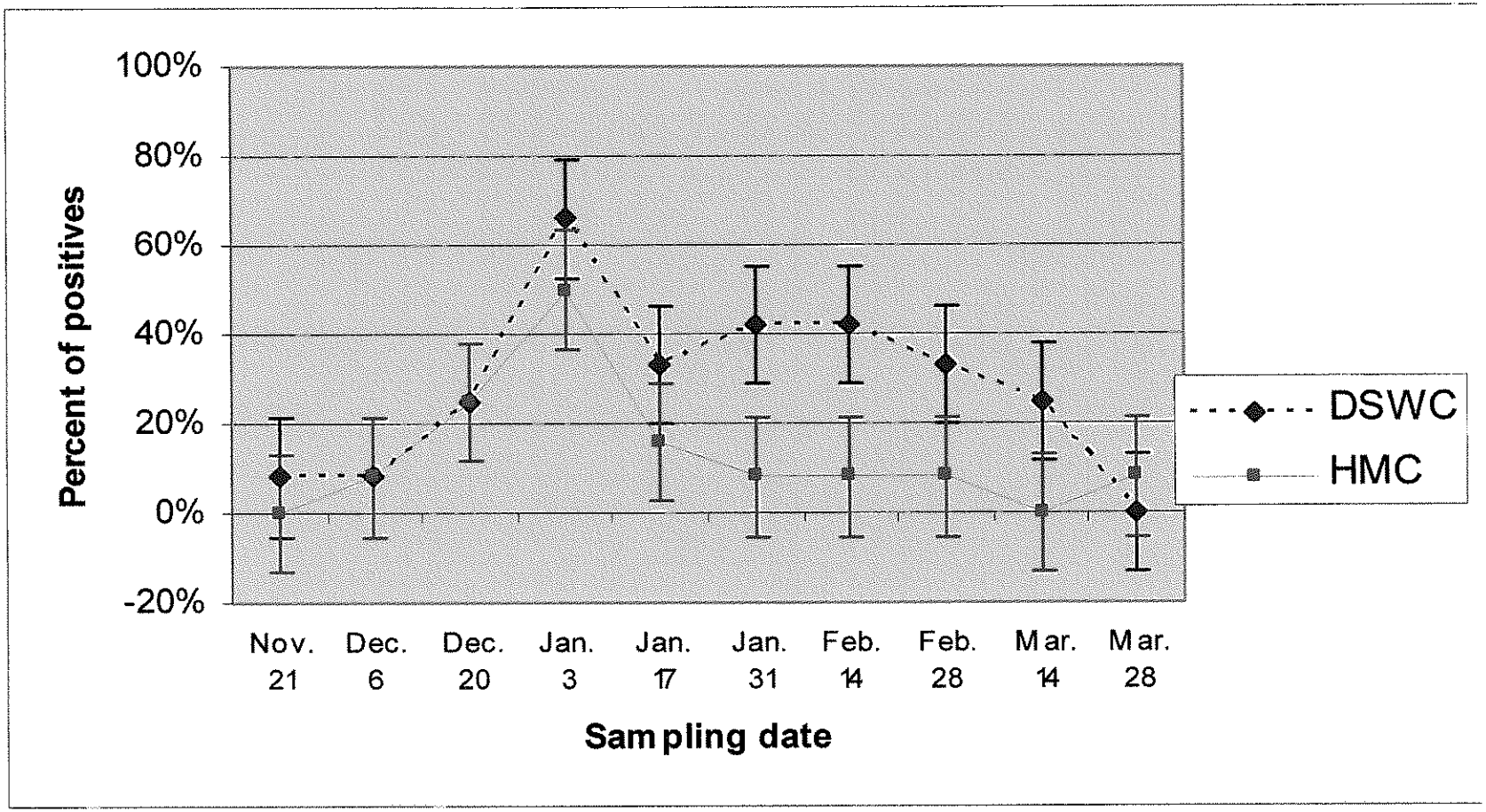


Figure 4.9: Percentage of pens for each energy source, dried whole-shelled corn (DSWC) or high-moisture corn (HMC), at each sampling period that contained at least one high shedder of *E. coli* O157:H7 as determined by direct plating on $SMAC_{CT}$ plates.

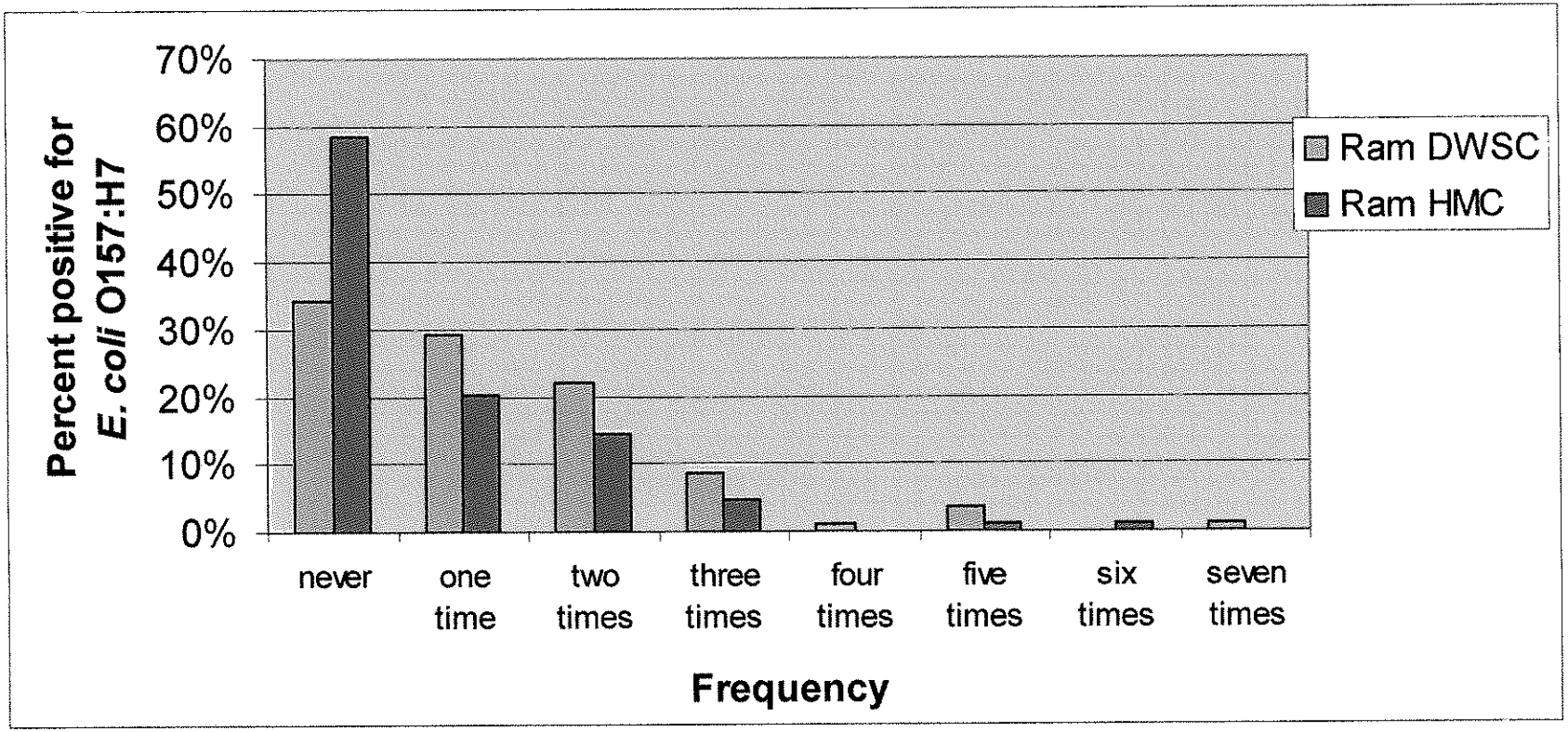


Figure 4.10: The frequencies in which cattle being fed different energy sources, dried whole-shelled corn (DSWC) or high-moisture corn (HMC), were positive for *E. coli* O157:H7 during the finishing period as determined by rectoanal mucosal swabs.

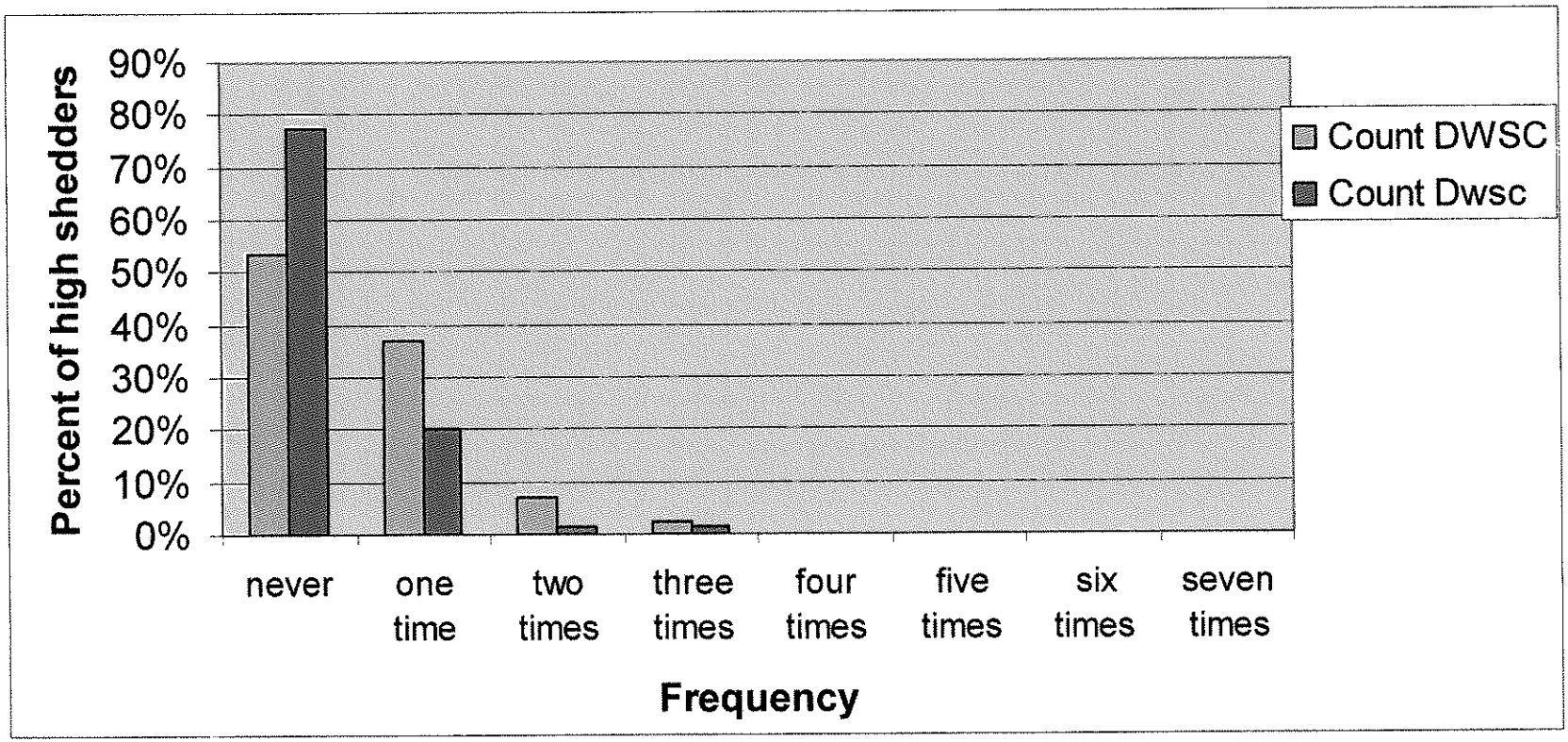


Figure 4.11: The frequencies in which cattle being fed different energy sources, dried whole-shelled corn (DSWC) or high-moisture corn (HMC), were determined to be excreting high amounts of *E. coli* O157:H7 during the finishing period as determined by direct plating on $SMAC_{CT}$ plates.

LIST OF REFERENCES

Akin, D. E. 1986. Interaction of ruminal bacteria and fungi with Southern forages. J. Anim. Sci. 63:962.

Akin, D. E., and L. L. Rigsby. 1987. Mixed fungal populations and lignocellulosic tissue degradation in the bovine rumen. Appl. Environ. Microbiol. 53:1987.

Akin, D. E. 1989. Histological and physical factors affecting digestibility of forages. Agron. J. 81:17.

Akin, D. E., and W. S. Borneman. 1990. Role of rumen fungi in fiber digestion. J. Dairy Sci. 73:3023-3032.

Anderson, R. C., S. A. Buckley, L. F. Kubena, L. H. Stanker, R. B. Harvey, and D. J. Nisbet. 2000. Bactericidal effect of sodium chlorate on *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT104 in rumen contents in vitro. J. Food Protect. 63:1038-1042.

AOAC. 1984. Official Methods of Analysis 14th ed. Association of Official Analytical Chemists, Washington, D.C.

Arcos-Garcia, J. L., F. A. Castrejon, G. D. Mendoza, and E. P. Perez-Gavilan. 2000. Effect of two commercial yeast cultures with *Saccharomyces cerevisiae* on ruminal fermentation and digestion in sheep fed sugar cane tops. Livestock Prod. Sci. 63:153-157.

Bach, S. J., T. A. McAllister, D. M. Veira, V. P. J. Gannon, and R. A. Holley. 2002a. Evaluation of bacteriophage DC22 for control of Escherichia coli O157:H7. J. Anim. Sci. 80(Suppl. 1):262. (Abstr.)

Bach, S. J., T. A. McAllister, D. M. Veira, V. P. J. Gannon, and R. A. Holley. 2002b. Effect of monensin on survival and growth of *Escherichia coli* O157:H7 in vitro. Can. Vet. J. 43:718-719.

Bacon, R. T., J. N. Sofos, K. E. Belk, D. R. Hyatt, and G. C. Smith. 2002. Prevalence and antibiotic susceptibility of *Salmonella* isolated from beef animal hides and carcasses. J. Food Protect. 65:284-290.

Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. J. Food Protect. 66:1978-1986.

Barkate, M. L., G. R. Acuff, L. M. Lucia, and D. S. Hale. 1993. Hot water decontamination of beef carcasses for reduction of initial bacterial numbers. Meat Sci. 35:397-401.

Beach, J. C., E. A. Murano, and G. R. Acuff. 2002. Prevalence of Salmonella and Campylobacter in beef cattle from transport to slaughter. J. Food Protect. 65:1687-1693.

Beharka, A. A., T. G. Nagaraja, and J. L. Morrill. 1991. Performance and ruminal function development of young calves fed diets with *Asperigillus oryzae* fermentation extract. J. Dairy Sci. 74:4326-4336.

Beharka, A. A., and T. G. Nagaraja. 1993. Effect of *Aspergillus oryzae* fermentation extract (Amaferm®) on in vitro fiber degradation. J. Dairy Sci. 76:812-818.

Beharka, A. A., and T. G. Nagaraja. 1998. Effect of *Aspergillus oryzae* extract alone or in combination with antimicrobial compounds on ruminal bacteria. J. Dairy Sci. 81:1591-1598.

Bell, B. P., M. Goldoft, P. Griffin, M. A. Davis, D. C. Gordon, P. I. Phillip, C. A. Bartleson, J. H. Lewis, T. J. Barrett, J. G. Wells, R. Baron, and J. Kobayashi. 1994. A multistate outbreak of *Escherichia coli* O157:H7-Associated bloody diarrhea and hemolytic uremic syndrome from hamburgers: The Washington experience. J. Amer. Med. Assoc. 272(17):1349-1353.

Berg, J., T. McAllister, S. Bach, R. Stilborn, D. Hancock, and J. LeJeune. 2004. *Escherichia coli* O157:H7 excretion by commercial feedlot cattle fed either barley- or corn-based finishing diets. J. Food Protect. 67:666-671.

Braden, K. W., J. R. Blanton, Jr., V. G. Allen, K. R. Pond, and M. F. Miller. 2004. Ascophyllum nodosum supplementation: A preharvest intervention for reducing *Escherichia coli* O157:H7 and *Salmonella* spp. in feedlot steers. J. Food Protect. 67:1824-1828.

Brannon, C. 2003. Prebiotics: Feeding friendly bacteria. Today's Dietician. September:12.

Brashears, M. M., M. L. Galyean, G. H. Loneragan, J. E. Mann, and K. K. Killinger Mann. 2003. Prevalence of *Escherichia coli* O157:H7 and performance by beef feedlot cattle given *Lactobacillus* direct-fed microbials. J. Food Protect. 66:748-754.

Brown, C. A., B. G. Harmon, T. Zhao, and M. P. Doyle. 1997. Experimental *Escherichia coli* O157:H7 carriage in calves. Appl. Environ. Microbiol. 63:27-32.

Buchko, S. J., R. A. Holley, W. O. Olson, V. P. J. Gannon, and D. M. Veira. 2000a. The effect of different grain diets on fecal shedding of *Escherichia coli* O157:H7 by steers. J. Food Protect. 63:1467-1474.

Buchko, S. J., R. A. Holley, W. O. Olson, V. P. J. Gannon, and D. M. Veira. 2000b. The effect of fasting and diet on fecal shedding of *Escherichia coli* O157:H7 by cattle. Can. J. Anim. Sci. 80:741-744.

Callaway, T. R., R. C. Anderson, K. J. Genovese, T. L. Poole, T. J. Anderson, J. A. Byrd, L. F. Kubena, and D. J. Nisbet. 2002a. Sodium chlorate supplementation reduces *E. coli* O157:H7 populations in cattle. J. Anim. Sci. 80:1683-1689.

Callaway, T., T. Edrington, R. Anderson, Y. S. Jung, K. Genovese, R. Elder, D. Nisbet. 2002b. Isolation of naturally-occurring bacteriophage from sheep that reduce populations of *E. coli* O157:H7 in vitro and in vivo. Int. Symp. On Shiga Toxin-Producing *Escherichia coli* Infections, Edinburgh, U.K.

Callaway, T. R., T. S. Edrington, R. C. Anderson, K. J. Genovese, T. L. Poole, R. O. Elder, J. A. Byrd, K. M. Bischoff, and D. J. Nisbet. 2003a. *Escherichia coli* O157:H7 populations in sheep can be reduced by chlorate supplemention. J. Food Protect. 66:194-199.

Callaway, T. R., R. O. Elder, J. E. Keen, R. C. Anderson, and D. J. Nisbet. 2003b. Forage feeding to reduce preharvest *Escherichia coli* populations in cattle, a review. J. Dairy Sci. 86:852-860.

Callaway, T. R., R. C. Anderson, T. S. Edrington, K. J. Genovese, K. M. Bischoff, T. L. Poole, Y. S. Jung, R. B. Harvey, and D. J. Nisbet. 2004. What are we doing about *Escherichia coli* O157:H7 in cattle. J. Anim. Sci. 82(E. Suppl.):E93-E99.

Castillo, A., L. M. Lucia, K. J. Goodson, J. W. Savell, and G. R. Acuff. 1998. Comparison of water wash, trimming, and combined hot water and lactic acid treatments for reducing bacteria of fecal origin on beef carcasses. J. Food Protect. 61:823-828.

CDC. 1995. Outbreak of Salmonella sereotype Typhimurium infection associated with eating raw ground beef—Wisconsin, 1994. Morbidity and Mortality Weekly Report 44(49):905-909.

CDC. 1996. Outbreak of Escherichia coli O157:H7 infection—Georgia and Tennessee, June 1995. Morbidity and Mortality Weekly Report 45(12):249-251.

CDC. 1997. Escherichia coli O157:H7 infections associated with eating nationally distributed commercial brand frozen ground beef patties and burgers—Colorado, 1997. Morbidity and Mortality Weekly Report 46(33):777-778.

Chang, J. S., E. M. Harper, and R. E. Calza. 1999. Fermentation extract effects on the morphology and metabolism of the rumen fungus *Neocallimastix frontalis* EB 188. J. Appl. Microbiol. 86:389-398.

Chesson, A., and C. W. Forseberg. 1988. Polysaccharide degradation by rumen microorganisms. In: P. N. Hobson (Ed.) The Rumen Microbial Ecosystem. p 77. Elsevier Science Publishing, New York..

Collins, M. D., and G. R. Gibson. 1999. Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. Am. J. Clin. Nutr. 69(suppl):1052S-1057S.

Couvaras, S., H. P. Van Niekerk, and S. E. Thomas. 1980. Effect of dietary lasalocid on coccidial oocyst numbers, feedlot performance and wool growth of lambs. J. S. Afr. Vet. Assoc. 51:111-113.

Dargatz, D. A., S. J. Wells, L. A. Thomas, D. D. Hancock, and L. P. Garber. 1997. Factors associated with the presence of *Escherichia coli* O157 in feces of feedlot cattle. J. Food Protect. 60:466-470.

Dargatz, D. A., P. J. Fedorka-Cray, S. R. Ladely, C. A. Kopral, K. E. Ferris, and M. L. Headrick. 2003. Prevalence and antimicrobial susceptibility of *Salmonella* spp. isolates from US cattle in feedlots in 1999 and 2000. J. Appl. Microbiol. 959(4):753-761.

Dawson, K. A., K. E. Newman, and J. A. Boling. 1990. Effects of microbial supplements containing yeast and Lactobacilli on roughage-fed ruminal microbial activities. J. Anim. Sci. 68:3392-3398.

Dehority, B. A. 1991. Cellulose digestion in ruminants. Pages 327-354 *in* Biosynthesis and Biodegradation of Cellulose. B. H. Haigle and P. J. Weimer, ed. Marcel Dekker, Inc., New York, NY.

Diez-Gonzalez, F., T. R. Callaway, M. G. Kizoulis, and J.B. Russell. 1998. Grain feeding and the dissemination of acid-resistance *Escherichia coli* from cattle. Science. 281:1666-1668.

Dorsa, W. J., C. N. Cutter, G. R. Sirgusa, and M. Koohmarie. 1996. Microbial contamination of beef and sheep carcasses by steam, hot water spray washes, and a steam-vacuum sanitizer. J. Food Protect. 59:127-135.

Dorsa, W. J., C. N. Cutter, and G. R. Siragusa. 1997. Effects of acetic acid, lactic acid, and trisodium phosphate on the microflora of refrigerated beef carcass surface tissue inoculated with *Escherichia coli* O157:H7, *Listeria innocua*, and *Clostridium sporogenes*. J. Food Protect. 60:619-624.

Edrington, T. S., T. R. Callaway, K. M. Bischoff, K. J. Genovese, R. O. Elder, R. C. Anderson, and D. J. Nisbet. 2003a. Effect of feeding ionophores monensin and laidlomycin propionate and the antimicrobial bambermycin to sheep experimentally infected with *E. coli* O157:H7 and *Salmonella typhimurium*. J. Anim. Sci. 81:553-560.

Edringon, T. S., T. R. Callaway, R. C. Andereson, K. J. Genovese, Y. S. Jung, J. L. McReynolds, K. M. Bischoff, and D. J. Nisbet. 2003b. Reduction of *E. coli* O157:H7 populations in sheep by supplementation of an experimental sodium chlorate product. Sm. Anim. Res. 49:173-181.

Elam, N. A., J. F. Gleghorn, J. D. Rivera, M. L. Galyean, P. J. Defoor, M. M. Brashears, and S. M. Younts-Dahl. 2003. Effects of live cultures of *Lactobacillus acidophilus* (strains NP45 and NP51) and *Propionbacterium freudenreichii* on performance, carcass, and intestinal characteristics, and *Escherichia coli* strain O157 shedding of finishing beef steers. J. Anim. Sci. 81:2686-2698.

Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmarie, and W. W. Laegreid. 2000. Correlation of enterhemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Appl. Biol. Sci. 97:2999-3003.

Elder, R. O., J. E. Keen, T. E. Wittum, T. R. Callaway, T. S. Edrington, R. C. Anderson, and D. J. Nisbet. 2002. Intervention to reduce fecal shedding of enterohemorrhagic *Escherichia coli* O157:H7 in naturally infected cattle using neomycin sulfate. J. Anim. Sci. 80:151 (Suppl. 1).

Fegan, N., P. Vanderlinde, G. Higgs, and P. Desmarchelier. 2004. Quantification and prevalence of Salmonella in beef cattle presenting at slaughter. J. Appl. Microbiol. 97:892-898.

Fegan, N., P. Vanderlinde, G. Higgs, and P. Desmarchelier. 2005. A study of the prevalence and enumeration of *Salmonella enterica* in cattle and on carcasses during processing. J. Food Protect. 68:1147-1153.

Ferrell, C. L., L. J. Koong, and J. A. Nienaber. 1986. Effect of previous nutrition on body composition and maintenance energy costs of growing lambs. Br. J. Nutr. 56:595-605.

Fluharty, F. L., K. E. McClure, M. B. Solomon, D. D. Clevenger, and G. D. Lowe. 1999. Energy source and ionophore supplementation effects on lamb growth, carcass characteristics, visceral organ mass, diet digestibility, and nitrogen metabolism. J. Anim. Sci. 77:816-823.

Fuller, R. 1989. A review: Probiotics in man and animals. J. Appl. Bacteriol. 66:365-378.

Funk, J. A., P. R. Davies, and M. A. Nichols. 2000. The effect of fecal sample weight on the detection of *Salmonella enterica* in swine feces. J. Vet. Diag. Invest. 12:412-418.

Funk, J. A., P. R. Davies, and M. A. Nichols. 2001. Longitudinal study of *Salmonella enterica* in growing pigs reared in multiple site production systems. Vet. Microbiol. 83:45-60.

Galland, J. C., D. R. Hyatt, S. S. Crupper, and D. W. Acheson. 2001. Prevalence, antibiotic susceptibility and diversity of *Escherichia coli* O157:H7 isolates from a longitudinal study of beef cattle feedlots. Appl. Environ. Microbiol. 67:1619-1627.

Garcia, C. C. G., M. G. D. Mendoza, M. S. Gonzalez, P. M. Cobos, C. M. E. Ortega, and L. R. Ramirez. 2000. Effect of a yeast culture (*Sacchromyces cerevisiae*) and monensin on ruminal fermentation and digestion in sheep. Anim. Feed Sci. Tech. 83:165-170.

Gedek, B. R. 1999. Adherence of *Escherichia coli* serogroup O157 and the *Salmonella Typhimurium* mutant DT 104 to the surface of *Saccharomyces boulardii*. Mycoses. 42:261-264.

Gomez-Alarcon, R. A., C. Dudas, and J. T. Huber. 1990. Influence of cultures of *Aspergillus oryzae* on rumen and total tract digestibility of dietary components. J. Dairy Sci. 73:703-710.

Goodrich, R. D., J. E. Garrett, D. R. Gast, M. A. Kirick, D. A. Larson, and J. C. Meiske. 1984. Influence of monensin on the performance of cattle. J. Anim. Sci. 58:1484-1498.

Gorden, J., and P. L. Small. 1993. Acid resistance in enteric bacteria. Infect. Immun. 61:364-367.

Grauke, L. J., I. T. Kudva, J. W. Yoon, C. W. Hunt, C. J. Williams, and C. J. Hodve. 2002. Gastrointestinal tract location of *Escherichia coli* O157:H7 in ruminants. Appl. Environ. Microbiol. 68:2269-2277.

Griffin, P. M. and R. V. Tauxe. 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemmorhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol. Rev. 13:60-98.

Hancock, D. D., D. H. Rice, L. A. Thomas, D. A. Dargatz, and T. E. Besser. 1997. Epidemiology of *Escherichia coli* O157 in feedlot cattle. J. Food Protect. 60:462-465.

Hardin, M. D., G. R. Acuff, L. M. Lucia, J. S. Oman, and J. W. Savell. 1995. Comparison of methods for contamination removal from beef carcass surfaces. J. Food Protect. 58:368-374.

Harper, E. G., R. P. Welch, D. C. Lara, J. S. Chang, and R. E. Calza. 1996. The effect of *Aspergillus oryzae* fermentation extract on the anaerobic fungi *Neocallimastix frontalis* EB 188, *Piromyces cocmmunis* DC 193 and *Orpinomyces* ssp. RW 206: generalized effects and component analysis. Appl. Environ. Biotechnol. 45:817-821.

Hovde, C. J., P. R. Austin, K. A. Cloud, C. J. Williams, and C. W. Hunt. 1999. Effect of cattle diet on *Escherichia coli* O157:H7 acid resistance. Appl. Environ. Microbiol. 65:3233-3235.

Huck, G. L., K. K. Kreikemeier, G. L. Kuhl, T. P. Eck, and K. K. Bolsen. 1998. Effects of feeding combinations of steam-flaked grain sorghum and steam-flaked, high-moisture, or dry-rolled corn on growth performance and carcass characteristics in feedlot cattle. J. Anim. Sci. 76:2984-2990.

Hungate, R. E., R. W. Dougherty, M. P. Bryant, and R. M. Cello. 1952. Microbiological and physiological changes associated with acute indigestion in sheep. Cornell Vet. 42:423-449.

Jay, J. M., M. J. Loessner, D. A. Golden. 2005. Modern Food Microbiology. Page 620-621 in Foodborne Gastroenteritis Caused by Salmonella and Shigella. 7th ed. Springer Science+Business Media, Inc., New York, NY.

Keen, J. E., G. A. Uhlich, and R. O. Elder. 1999. Effects of hay and grain-based diets on fecal shedding of naturally acquired enterohemmorhagic Escherichia coli (Ehec) O157 in beef feedlot cattle. 80th Conference Research Workers in Animal Diseases, Nov. 7-9, Chicago, IL.

Khaitsa, M. L., D. R. Smith, J. A. Stoner, A. M. Parkhurst, S. Hinkley, T. J. Klopfenstein, and R. A. Moxley. 2003. Incidence, duration, and prevalence of *Escherichia coli* O157:H7 fecal shedding by feedlot cattle during the finishing period. J. Food Protect. 66:1972-1977.

Khaitsa, M. L., M. L. Bauer, P. S. Gibbs, G. P. Lardy, D. Doetkott, and R. B. Kegode. 2005. Comparison of two sampling methods for *Escherichia coli* O157:H7 detection in feedlot cattle. J. Food Protect. 68:1724-1728.

Kochevar, S. L., J. N. Sofos, R. R. Bolin, J. O. Reagan, and G. C. Smith. 1997. Steam vacuuming as a pre-evisceration intervention to decontaminate beef carcasses. J. Food Protect. 60:107-113.

Krehbiel, C. R., S. R. Rust, G. Zhang, and S. E. Gilliland. 2003. Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. J. Anim. Sci. 81(E. Suppl. 2):E120.

Kudva, I. T., P. G. Hatfield, and C. J. Hovde. 1996. *Escherichia coli* O157:H7 in microbial flora of sheep. J. Clin. Microbiol. 34:431-433.

Kudva, I. T., C. W. Hunt, C. J. Williams, U. M. Nance, and C. J. Hovde. 1997. Evaluation of dietary influences on *Escherichia coli* O157:H7 shedding by sheep. Appl. Environ. Microbiol. 63:3878-3886.

Kvietkute, N., R. Gruzauskas, A. Raceviciute-Stupeliene, and V. Sasyte. 2005. Effect of probiotic Levucell SB on growth rate in suckling and weaned pigs. Veterinarija IR Zootechnika. 32(54).

Ladley, S. R., R. A. Stock, F. K. Goedeken, and R. P. Huffman. 1995. Effect of corn hybrid and grain processing method on rate of starch disappearance and performance of finishing cattle. J. Anim. Sci. 73:360-364.

LeJeune, J. T., T. E. Besser, D. H. Rice, J. L. Berg, R. P. Stilborn, and D. D. Hancock. 2004. Longitudinal study of fecal shedding of *Escherichia coli* O157:H7 in feedlot cattle: Predominance and persistence of specific clonal types despite massive cattle population turnover. Appl. Environ. Microbiol. 70:377-384.

Lema, M., L. Williams, and D. R. Rao. 2001. Reduction of fecal shedding of enterohemorrhagic *Escherichia coli* O157:H7 in lambs by feeding microbial feed supplement. Sm. Rum. Res. 39:31-39.

Loneragan, G. H. and M. M. Brashears. 2005. Pre-harvest interventions to reduce carriage of *E. coli* O157:H7 by harvest-ready feedlot cattle. Meat Sci. 71:72-78.

Martin, S. A., and D. J. Nisbet. 1990. Effects of *Aspergillus oryzae* fermentation extract on fermentation of amino acids, bermudagrass and starch by mixed ruminal microorganisms in vitro. J. Anim. Sci. 68:2142-2149.

McAllister, T. A., H. D. Bae, G. A. Jones, and K. J. Cheng. 1994. Microbial attachment and feed digestion in the rumen. J. Anim. Sci. 72:3004-3018.

McEvoy, J. M., A. M. Doherty, J. J. Sheridan, I. S. Blair, and D. A. McDowell. 2003. The prevalence of *Salmonella* spp. in bovine faecal, rumen and carcass samples at a commercial abattoir. J. Appl. Microbiol. 94:693-700.

Mead, P. S., L. Slutsker, V. Dietz, L. F. McCraig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxee. 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. 5:607-625.

Meyer-Broseta, S., S. N. Bastian, P. D. Arne, O. Cerf, and M. Sanaa. 2001. Review of epidemiological surveys on the prevalence of contamination of healthy cattle with *Escherichia coli* serogroup O157:H7. Int. J. Hyg. Environ. Health 203:347-361.

Mies, P. D., B. R. Covington, K. B. Harris, L. M. Lucia, G. R. Acuff, and J. W. Savell. 2004. Decontamination of cattle hides prior to slaughter using washes with and without antimicrobial agents. J. Food Protect. 67:579-582.

Murphy, T. A., S. C. Loerch, K. E. McClure, and M. B. Solomon. 1994. Effects of grain or pasture finishing systems on carcass composition and tissue accretion rates of lambs. J. Anim. Sci. 72:3138-3144.

Naylor, S. W., C. Low, T. E. Besser, A. Mahajan, G. J. Gunn, M. C. Pearce, I. J. McKendrick, D. G. E. Smith, and D. L. Gally. 2003. Lymphoid follicule-dense mucosa at the terminal rectum is the principal site of enterohemorrhagic *E. coli* O157:H7 in the bovine host. Infection and Immunity 71:1505-1512.

Newbold, C. J., R. J. Wallace, X. B. Chen, and F. M. McIntosh. 1995. Different strains of *Saccharomyces cerevisiae* differ in their effects on ruminal bacterial numbers in vitro and in sheep. J. Anim. Sci. 73:1811-1818.

Newman, K. E., K. A. Dawson, and M. C. Morehead. 1990. Antagonistic activities of bacterial isolates from probiotic feed supplements upon pathogenic and rumen bacteria. J. Anim. Sci. 68 (Suppl. 1):505 (Abstr.).

Nisbet, D. J., and S. A. Martin. 1990. Effect of dicarboxylic acids and *Aspergillus oryzae* fermentation extract on lactate uptake by the ruminal bacterium *Selenamonas ruminantium*. Appl. Environ. Microbiol. 56:3515-3518.

Nisbet, D. J., and S. A. Martin. 1991. Effect of *Saccharomyces cerevisiae* culture on lactate utilization by the ruminal bacterium *Selenomonas ruminatium*. J. Anim. Sci. 69:4628-4633.

NRC. 1985. Nutrient Requirements of Sheep (6th Ed.). National Academy Press, Washington, DC.

Nutsch, A. L., R. K. Phebus, M. J. Riemann, D. E. Schafer, J. E. Boyer Jr., R. C. Wilson, J. D. Leising, and C. L. Kastner. 1997. Evaluation of a steam pasteurization process in a commercial beef processing facility. J. Food Protect. 60:485-492.

Owens, F. N., D. S. Secrist, W. J. Hill, and D. R. Gill. 1998. Acidosis in cattle: A review. J. Anim. Sci. 76:275-286.

Potter, A. A., S. Klashinsky, Y. Li, E. Frey, H. Townsend, D. Rogan, G. Erickson, S. Hinkley, T. Klopfenstein, R. A. Moxley, D. R. Smith, and B. B. Finlay. 2004. Decreased shedding of *Escherichia coli* O157:H7 by cattle following vaccination with type III secreted proteins. Vaccine 22:362-369.

Rasmussen, M. A., T. L. Wickman, W. C. Cray Jr., and T. A. Casey. 1999. *Escherichia coli* O157:H7 and the rumen environment. Page 39 in *E. coli* O157 in Farm Animals. C. S. Stewart and H. J. Flint, ed. CAB Int, New York.

Russeell, J. B. and A. J. Houlihan. 2003. Ionophore resistance of ruminal bacteria and its potential impact on human health. Microbiol. Rev. 27:65-74.

SAS. (1999). SAS User's Guide: Statistics. SAS institiute Inc. Cary, NC.

Schelling, G. T. 1984. Monensin mode of action in the rumen. J. Anim. Sci. 58:1518-1527.

Schmidt, J. A., S. Albright, K. P. Tsai, G. M. Calza, J. S. Chang, and R. E. Calza. 2004. Characterization of *Aspergillus oryzae* fermentation extract effects on the rumen fungus *Neocallimastix frontalis* EB 188. Part 1. Zoospore development and physiology. App. Microbiol. Biotechnol. 63:422-430.

Slyter, L. L. 1976. Influence of acidosis on rumen function. J. Anim. Sci. 43:910-929.

Smith, M. G. and A. Graham. 1978. Destruction of *Escherichia coli* and *Salmonellae* on mutton carcasses by treatment with hot water. Meat Sci. 2:119-128.

Swanson, K. S., C. M. Grieshop, E. A. Flickinger, L. L. Bauer, H. Healy, K. A. Dawson, N. R. Merchen, and G. C. Fahey Jr. 2002. Supplemental fructooligosacchrides and mannanoligosaccharides influence immune function, ileael and total tract nutrient digestibilities, microbial populations and concentrations of protein catabolites in the large bowel of dogs. Am. Soc. Nutr. Sci. 132:980-989.

Tkalcic, S., C. A. Brown, B. G. Harmon, A. V. Jain, P. O. Eric, A. Parks, K. L. Jacobsen, S. A. Martin, T. Zhao, and M. P. Doyle. 2000. Effects of diet on rumen proliferation and fecal shedding of *Escherichia coli* O157:H7 in calves. J. Food Protect. 63:1630-1636.

Van Baale, M. J., J. M. Sargeant, D. P. Gnad, B. M. DeBey, K. F. Lechtenberg, and T. G. Nagaraja. 2004. Effect of forage and grain diet with or without monensin on ruminal persistence and fecal *Escherichia coli* O157:H7 in cattle. Appl. Environ. Microbiol. 70:5336-5342.

Van Donkersgoed, J., D. Hancock, D. Rogan, A. A. Potter. 2005. *Escherichia coli* O157:H7 vaccine field trail in 9 feedlots in Alberta and Saskatchewan. Can. Vet. J. 46:724-728.

Varel, V. H. and K. K. Kreikemeier. 1994. Influence of feeding *Aspergillus oryzae* fermentation extract (Amaferm) on in situ fiber degradation, ruminal fermentation, and microbial protein synthesis in nonlactating cows fed alfalfa or bromegrass hay. J. Anim. Sci. 72:1814-1822.

Waldrip, H. M., and S. A. Martin. 1993. Effect of an *Aspergillus oryzae* fermentation extract and other factors on lactate utilization by the ruminal bacterium *Megasphaera elsdenii*. J. Anim. Sci. 71:2770-2776.

Walker, W. A., and L. C. Duffy. 1998. Diet and bacterial colonization: Role of probiotics and prebiotics. J. Nutr. Biochem. 9:668-675.

Weir, E. 2000. *Escherichia coli* O157:H7. Canadian Med. Asssoc. J. 163(2):205.

Yoon, I. K., and M. D. Stern. 1995. Influence of direct-fed microbials on ruminal microbial fermentation and performance of ruminants: A review. Asian-Australas. J. Anim. Sci. 8:533-555.

Yoon, I. K., and M. D. Stern. 1996. Effect of *Saccharomyces cerevisiae* and *Aspergillus oryzae* cultures on ruminal fermentation in dairy cows. J. Dairy Sci. 79:411-417.

Younts-Dahl, S. M., M. L. Galyean, G. H. Loneragan, N. A. Elam, and M. M. Brashears. 2004. Dietary supplementation with *Lactobacillus*- and *Propionbacterium*-based direct-fed microbials and prevalence of *Escherichia coli* O157 in beef feedlot cattle and on hides at harvest. J. Food Protect. 67:889-893.

Younts-Dahl, S. M., G. D. Osborn, M. L. Galyean, J. D. Rivera, G. H. Loneragan, and M. M. Brashears. 2005. Reduction of *Escherichia coli* O157 in finishing beef cattle by various doses of *Lactobacillus acidophilus* in direct-fed microbials. J. Food Protect. 68:6-10.