FEEDING A DIRECT-FED MICROBIAL TO DETERMINE PERFORMANCE, CARCASS CHARACTERISTICS, AND FECAL SHEDDING OF *ESCHERICHIA COLI* O157:H7 IN FEEDLOT HEIFERS FED WITH OR WITHOUT WET DISTILLER'S GRAINS PLUS SOLUBLES

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NOMENCLATURE

AAFCO	Association of American Feed Control Officials
ADG	Average Daily Gain
BW	Body Weight
DFM	Direct-Fed Microbial
DM	Dry Matter
DMI	Dry Matter Intake
DRC	Dry-Rolled Corn
FDA	Food and Drug Administration
G:F	Average Daily Gain:Dry Matter Intake
HCW	Hot Carcass Weight
KPH	Kidney Pelvic and Heart Fat
NE_m	Net Energy Required for Maintenance
NE_g	Net Energy Required for Gain
WDGS	Wet Distiller's Grains plus Solubles

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CHAPTER I

INTRODUCTION

The production of biofeuls in the United States has dramatically increased in recent years. In 1980, 662,447,065 liters (175,000,000 gallons) of ethanol were produced. In 2009, production had increased to 40,693,176,850 liters (10,750,000,000 gallons) (RFA, 2010a). Ethanol production rose 232% between 2003 and 2007 (USDA, ERS, 2009). This increased ethanol production can have a drastic impact on commodity prices and agricultural profitability (CAST, 2006). This has become evident with greater demand for corn and higher corn prices. Increasing corn prices have had a significant impact on the cost of gain for cattle producers who rely heavily on corn-based diets. Currently, grain-based ethanol is the only viable source of biofuel in the United States (CAST, 2006). The production of ethanol yields several byproducts or co-products, and with increased production by the ethanol industry, a substantial amount of these byproducts are available. Some of these byproducts have provided the cattle industry with new viable feed options. One such byproduct, wet distiller's grains, contains valuable nutrients and can be incorporated into rations for cattle (USDA, ERS, 2009). The inclusion of wet distiller's grains in feedlot diets has become a common practice in many regions of the country. Wet distiller's grains plus solubles (WDGS) has demonstrated a greater energy value and improved cattle performance compared to both

dry-rolled and high-moisture corn (Ham et al., 1994; Lodge et al., 1997; Klopfenstein et al., 2008). It has been observed that the optimum inclusion level for WDGS in feedlot diets is between 30 and 40% of diet dry matter (Vander Pol et al., 2006b). Most research conducted with dry-rolled and high-moisture corn-based diets indicate that inclusion of up to 30% wet distiller's grains in the diet has no negative impact on finishing performance and carcass characteristics.

While there are several advantages to feeding wet distiller's grains, one possible disadvantage of feeding them is an increased incidence of *Escherichia coli* O157:H7 shedding. Currently, there is inconsistent scientific evidence that distiller's grains, at the levels fed commercially, increase *E. coli* shedding (Klopfenstein et al., 2009). Conversely, some research has indicated that there is a connection between feeding distiller's grains and increased *E. coli* shedding in feedlot cattle (Jacob et al., 2008).

The feeding of direct-fed microbials (DFM) has received much consideration from the feedlot industry. There is a current perception that there is a need for sufficient disease prevention and enhanced performance with a reduction of antimicrobial use in livestock production (Krehbiel et al., 2003; Raeth-Knight et al., 2007). Direct-fed microbials have been a well received alternative since they contain a source of live, naturally occurring microorganisms (Yoon and Stern, 1995; AAFCO, 1999; FDA, 2003). Data suggest that DFM have the potential to improve production efficiency in feedlot cattle, alter ruminal fermentation processes and products, and decrease the shedding of harmful human pathogens (Yoon and Stern, 1995; Krehbiel et al., 2003; McDonald et al., 2005). A possible application for DFM is to reduce *E. coli* O157:H7 shedding in feedlot cattle. Numerous studies have demonstrated that feeding DFM to cattle decreases the

fecal shedding of *E. coli* O157:H7 (Brashears et al., 2003; Elam et al., 2003; Younts-Dahl et al., 2005; Tabe et al., 2008; Callaway et al., 2009).

The purpose of this experiment was to evaluate the effects of a direct-fed microbial containing *Lactobacillus acidophilus* combined with *Propionibacterium freudenreichii* on performance, carcass characteristics, and fecal shedding of *Escherichia coli* O157:H7 in feedlot heifers fed with or without wet distiller's grains plus solubles. It has been well documented that WDGS improves feedlot cattle performance. Although improved performance has been well established, some research indicates that feeding distiller's grains causes an undesirable increase in *E. coli* shedding in feedlot cattle. Data suggest that DFM have the potential to improve production efficiency and reduce *E. coli* O157:H7 shedding in cattle. We hypothesized that the inclusion of WDGS in the diet would improve performance and efficiency of heifers compared to a control corn-based diet, and that DFM would promote additional performance and efficiency while simultaneously preventing increases in the shedding of *E. coli* O157:H7 that could potentially occur due to the inclusion of WDGS in the diet.

CHAPTER II

REVIEW OF LITERATURE

History of Ethanol and Distiller's Grains and Their Involvement in the Feedlot Industry

Ethanol was first prepared synthetically by Henry Hennel and S. G. Serullas in 1826 (SPE, 2009). The first use of ethanol as engine fuel was in that same year by Samuel Morey, who invented an engine that ran on ethanol and turpentine (SPE, 2009). Early ethanol was used principally as a lighting fuel. Ethanol production was reduced dramatically during the Civil War, due to the implementation of a liquor tax (SPE, 2009). This tax caused ethanol production levels to remain low until the tax was repealed in 1906 (SPE, 2009). In 1908, Henry Ford designed his Model T to be a flexible fuel vehicle that could run on ethanol. The carburetors in the Model T could be adjusted to use alcohol, gasoline, or a "gasohol" mix (Solomon et al., 2007).

While feeding distiller's grains to livestock has become exceedingly popular in recent years, distiller's grains have an extensive history as a livestock feed. One of the earliest accounts observing the feeding of distiller's grains to cattle in the U.S. was published in 1900 in Feeds and Feeding (Henry, 1900). An additional early study of feeding distiller's grains to cattle was published in 1907 (Weiss et al., 2007). The next major decline in ethanol production was due to Prohibition in 1919 (SPE, 2009). Alcohol- based fuels experienced a resurgence in the 1930's due to farmers in the

Midwest seeking alternative uses for their corn due to falling corn prices (Solomon et al., 2007). In the late 1930's and early 1940's studies by Morrison and Garrigus and Good, as stated by Klopfenstein et al. (2008), refer to a wet form of byproduct feed called "distiller's slop" that was fed to beef cattle. In recent history, distiller's grains production in the United States has increased from 2.3 million metric tons in 1999 to 23.0 million metric tons in 2008, or the production of distiller's grains increased by 1000% in the last ten years alone (RFA, 2009). Increases in modern ethanol production in the U.S. have emerged principally as a result of actions of the government. The ethanol industry has been aided by numerous subsidies, tax exemptions, loans, and price guarantees (Solomon et al., 2007). As a result, the U.S. ethanol industry has experienced swift growth. In 2009 alone, 40,693,176,850 liters (10,750,000,000 gallons) of ethanol were produced (RFA, 2010a).

Current Production and Scope of Wet Distiller's Grains plus Solubles

Due to the rapid growth of the U.S. ethanol industry since 2002, there has been an equivalent explosion of growth in the production of ethanol co-products (Solomon et al., 2007; RFA, 2009). As a result of the recent increases in ethanol byproduct production, notably distiller's grains, there has been an enhanced interest in feeding these byproducts to livestock (Weiss et al., 2007). Besides increases in production, modern ethanol plants have greatly improved in efficiency. Today, an ethanol refinery can produce approximately 10.6 liters (2.8 gallons) of ethanol and over 7.7 kilograms (17 pounds) of distiller's grains from a single bushel of corn (RFA, 2009). The production of the 23.0 million metric tons of distiller's grains in 2008 was significantly important for ethanol

producers. The value of ethanol co-products utilized for livestock feed during 2007-2008 was estimated at $3 billion (RFA, 2009). The production of distiller's grains increased nearly 33% from 2008 to 2009. Total production in 2009 was approximately 30.5 million metric tons (RFA, 2010b). Exports alone in 2009 were 5.64 million metric tons (RFA, 2010b). This amount of exports is noteworthy as the level of distiller's grains exported in 2009 is equivalent to the total production of distiller's grains in 2003 (RFA, 2010b).

Utilization of Wet Distiller's Grains plus Solubles in Feedlots

While distiller's grains have an extensive history as a livestock feed, the use of wet distiller's grains in commercial feedlot diets is a relatively recent phenomenon. According to a survey of cattle feeders conducted by the National Agricultural Statistics Service (NASS), feedlots had utilized distiller's co-products in rations for only 5.1 years, on average (USDA, NASS, 2007). Despite being used as a widespread ration ingredient in feedlot diets in only modern years, wet distiller's grains offer several benefits for cattle feeders. When looking at all livestock operations, feedlots report paying discounted prices for distiller's grains compared to dairy, cow-calf, or swine operations, and feedlots can utilize distiller's grains with higher moisture content compared to these other operations (USDA, NASS, 2007). One of the reasons for the continued success of wet distiller's grains plus solubles in feedlots is due to the greater energy value of the wet distiller's grains plus solubles compared to both dry-rolled and high-moisture corn (Vander Pol et al., 2009). Several metabolism studies have suggested that the fat contained in distiller's grains is partially protected from degradation in the rumen (Klopfenstein et al., 2008). This could lead to a larger portion of the fat entering the

small intestine which would increase the total tract digestibility of fat. Distiller's grains have also been shown by several studies to be a substantial source of rumen undegradable intake protein (Klopfenstein et al., 2008). The ruminal undegradable fat along with the ruminal undegradable protein contained in distiller's grains may explain some of the greater feeding value of wet distiller's grains when compared to corn (Klopfenstein et al., 2008). The greater energy value of wet distiller's grains plus solubles observed may also be due to controlling subacute acidosis or overall increased energy utilization (Stock et al., 2000). There are definitely optimal levels for inclusion of wet distiller's grains plus solubles in feedlot diets. These optimal inclusion rates are dependent on animal nutrition and performance as well as economics. Klopfenstein et al. (2008) conducted a meta-analysis consisting of nine studies in which varying levels of wet distiller's grains plus solubles were fed. The inclusion rates of wet distiller's grains plus solubles ranged from 10% of diet dry matter to 50% of diet dry matter (Klopfenstein et al., 2008). In the meta-analysis, in addition to being suggested by other numerous researchers, the optimal inclusion rate of wet distiller's grains plus solubles in feedlot diets lies somewhere between 20% and 40% from an animal nutrition and performance standpoint (Vander Pol et al., 2006b; Weiss et al., 2007; Klopfenstein et al., 2008; Black, 2009; Vander Pol et al., 2009). When the level of wet distiller's grains in the ration exceeds 40%, animal performance has been diminished (Vander Pol et al., 2006b; Weiss et al., 2007; Klopfenstein et al., 2008; Black, 2009; Vander Pol et al., 2009). When looking at optimal inclusion rate from an economic perspective the inclusion rate varies according to several factors including, but not limited to: current corn prices, transportation costs,

distance from the plant, facilities, equipment, storage, and feeding capacity (Jones et al., 2007).

Effects of Wet Distiller's Grains plus Solubles on Cattle Performance

Animals fed wet distiller's grains plus solubles have demonstrated improved performance, and improving animal performance is vital to the success and profitability of the feedlot industry. Wet distiller's grains plus solubles have been shown to have greater feeding values and improved feed efficiency when compared to corn-based control diets (Vander Pol et al., 2006b; Klopfenstein et al., 2008; Black, 2009). In one study, wet distiller's grains plus solubles demonstrated feeding values between 121% and 178% of the feeding value of corn, depending upon inclusion rate in the diet (Vander Pol et al., 2006b). Research conducted at Iowa State University demonstrated feeding values for wet distiller's grains plus solubles of 140% to 180% that of corn (Loy, 2007). In a meta-analysis of nine studies in which varying levels of wet distiller's grains plus solubles were fed, the feeding values for the wet distiller's grains plus solubles were between 126% and 145% of the feeding value of corn on a dry matter basis (Klopfenstein et al., 2008). According to the same meta-analysis, diets containing wet distiller's grains plus solubles showed quadratic responses in average daily gain and dry matter intake with both being maximized at 20 to 30% wet distiller's grains plus solubles in the diet on a dry matter basis (Klopfenstein et al., 2008). Gain:feed had a linear effect and was maximized at 30 to 50% of the diet dry matter (Klopfenstein et al., 2008). Gain:feed also tended to be quadratic (Klopfenstein et al., 2008). As gain:feed values were not significant at the quadratic level, gain:feed never decreased with increasing wet distiller's

grains plus solubles in the diet, but tended to increase at a decreasing rate (Klopfenstein et al., 2008). However, due to accounting for the inclusion level in the diet, the feeding values calculated from the gain:feed values did decrease with increasing wet distiller's grains plus solubles in the diet (Klopfenstein et al., 2008). The meta-analysis demonstrated that the optimum level of wet distiller's grains to include in diets to maximize cattle performance lies somewhere between 20 and 30% for dry-rolled corn or high-moisture corn based diets (Klopfenstein et al., 2008). Elevated levels of wet distiller's grains in diets have shown a quadratic response in performance variables (Vander Pol et al., 2006b; Klopfenstein et al., 2008; Black, 2009). Nevertheless, cattle fed even higher than optimum levels of distiller's grains, up to 50% inclusion, have still shown numerically improved feed efficiency when compared to cattle on a control corn-based diet (Vander Pol et al., 2006b; Black, 2009).

Most of the studies comparing feeding values or energy content of wet distiller's grains plus solubles to corn have been conducted with diets that were not formulated to be isocaloric. This should be taken into consideration when evaluating the feeding value of wet distiller's grains plus solubles in diets. Distiller's grains contain a greater percentage of fat than the ingredients that are being replaced by the distiller's grains in the diet. To get an accurate feeding value comparison, the diets should be balanced for fat content to avoid large differences in the energy content of the diets being compared. This method results in reduced feeding values for diets containing wet distiller's grains plus solubles and a more realistic comparison to dry-rolled corn, high-moisture corn, or steam-flaked corn based diets. Leibovich et al. (2009) conducted an experiment evaluating corn processing method and sorghum wet distiller's grains plus solubles

inclusion where additional fat was included in the control diets. Steers fed the sorghum wet distiller's grains plus solubles had decreased gain:feed compared to steers fed dry-rolled corn or steam-flaked corn control diets. The decreased performance for the steers fed sorghum wet distiller's grains plus solubles resulted in lower calculated net energy for maintenance and net energy for gain values for the diets containing sorghum wet distiller's grains plus solubles (Leibovich et al., 2009). May et al. (2010) conducted an experiment where both corn and sorghum wet distiller's grains with solubles were fed in steam-flaked corn based diets. Final body weight, average daily gain, and carcass adjusted gain:feed were less for cattle fed wet distiller's grains plus solubles compared to cattle fed the control diets (May et al., 2010). No differences were observed in calculated net energy for maintenance and net energy for gain values for the average of diets containing distiller's grains plus solubles compared to the steam-flaked corn control diet (May et al., 2010). However, cattle fed corn wet distiller's grains with solubles or a blend of corn and sorghum wet distiller's grains with solubles had greater calculated net energy for maintenance and net energy for gain values compared to cattle fed only sorghum wet distiller's grains with solubles (May et al., 2010). These studies emphasize the importance of balancing diets for fat content when evaluating the energy value of dietary ingredients.

It is well established that wet distiller's grains plus solubles when fed at appropriate levels can improve cattle performance when compared to corn-based control diets (Klopfenstein et al., 2008). However, variations in performance and efficiency have been shown in cattle fed wet distiller's grains plus solubles depending on the grain utilized in the diet and the grain processing method (Vander Pol et al., 2006a;

Klopfenstein et al., 2008). In a trial reviewing three corn processing methods: dry-rolled corn, high-moisture corn, and steam-flaked corn with 30% wet distiller's grains plus solubles, cattle fed steam-flaked corn had decreased average daily gains when compared to the other two corn processing methods (Vander Pol et al., 2006a; Klopfenstein et al., 2008). Another study evaluated three corn processing methods, dry-rolled corn, high-moisture corn, and steam-flaked corn with increasing levels of wet distiller's grains plus solubles (Klopfenstein et al., 2008; Corrigan et al., 2009). A linear increase in gain:feed was shown with increasing wet distiller's grains plus solubles for both dry-rolled corn and high-moisture corn diets (Klopfenstein et al., 2008; Corrigan et al., 2009). However, there was no change in gain:feed with increasing wet distiller's grains plus solubles for the steam-flaked corn diet (Klopfenstein et al., 2008; Corrigan et al., 2009). Wet distiller's grains plus solubles has also been shown to affect molar proportions of acetate and propionate and the acetate to propionate ratio (Vander Pol et al., 2009). Feeding wet distiller's grains plus solubles tended to decrease acetate, increase propionate, and decrease the acetate to propionate ratio (Vander Pol et al., 2009).

Corn is the primary grain utilized for ethanol production, and as a result most wet distiller's grains plus solubles is derived from corn. However, grain sorghum has been and continues to be effectively utilized for ethanol production. Sorghum and corn have similar amounts of starch and therefore result in similar ethanol yields. Sorghum is generally less expensive than corn, making it an attractive option for ethanol plants (Klopfenstein et al., 2008). In a review of current research, Klopfenstein et al. (2008) evaluated 4 experiments in which feeding sorghum distiller's grains plus solubles was compared to corn distiller's grains plus solubles. The 4 experiments demonstrated no

significant differences in cattle performance between cattle fed sorghum distiller's grains plus solubles compared to corn distiller's grains plus solubles. However, Klopfenstein et al. (2008) suggested that corn distiller's grains plus solubles may be superior to sorghum distiller's grains plus solubles due to numerical differences in some of the trials.

In a study evaluating corn and sorghum distiller's byproduct digestibility in lambs, Lodge et al. (1997) concluded that corn wet distiller's grains were higher in true nitrogen digestibility, apparent nitrogen digestibility, and organic matter digestibility when compared to sorghum wet distiller's grains. In another experiment evaluating corn and sorghum wet distiller's grains in steers, average daily gain and feed efficiency were not different for diets containing sorghum wet distiller's grains or corn wet distiller's grains (Al-Suwaiegh et al., 2002). However, dry matter intake was greater for steers receiving sorghum wet distiller's grains compared to steers receiving corn wet distiller's grains (Al-Suwaiegh et al., 2002). Two additional studies conducted by Vasconcelos et al. (2007) and Depenbusch et al. (2009) directly compared diets containing sorghum wet distiller's grains to diets containing corn wet distiller's grains. Both Vasconcelos et al. (2007) and Depenbusch et al. (2009) observed that dry matter intake, averaged daily gain, and gain:feed were not different for diets containing sorghum wet distiller's grains compared to diets containing corn wet distiller's grains.

The research regarding sorghum wet distiller's grains is considerably more limited than that regarding corn wet distiller's grains, and the responses to different corn processing methods may be different than responses observed with corn wet distiller's grains. A trial evaluating corn processing method in diets containing sorghum wet distiller's grains plus solubles demonstrated no interaction between sorghum wet

distiller's grains plus solubles inclusion and corn processing method (Leibovich et al., 2009). Additionally, average daily gain and gain:feed were decreased with inclusion of sorghum wet distiller's grains plus solubles in the diet (Leibovich et al., 2009). This trial contradicts previous knowledge concerning sorghum wet distiller's grains plus solubles.

Effects of Wet Distiller's Grains plus Solubles on Carcass Merit, Meat Quality, and Sensory Attributes

Not only can wet distiller's grains affect animal performance, but they can also affect carcass characteristics. In a study evaluating three corn processing methods with increasing levels of wet distiller's grains plus solubles, wet distiller's grains plus solubles had significant linear or quadratic effects on hot carcass weight, 12^{th} rib fat thickness, marbling score, and yield grade (Corrigan et al., 2009). In the meta-analysis conducted by Klopfenstein et al. (2008) evaluating nine experiments in which wet distiller's grains plus solubles were fed at varying levels from 0 to 50% of diet dry matter, quadratic increases were observed for 12^{th} rib fat thickness and marbling scores. In addition, the meta-analysis showed the yield grades across the studies were linearly significant and tended to be quadratic (Klopfenstein et al., 2008). A second meta-analysis utilizing 21 different studies from 6 different states was conducted to evaluate carcass fat distribution of cattle fed various levels of distiller's grains (Reinhardt et al., 2007; Black, 2009). The meta-analysis observed that feeding low levels of distiller's grains (16% and lower) increased marbling score. In contrast, feeding high levels of distiller's grains (33% and higher) decreased marbling score (Reinhardt et al., 2007; Black, 2009). In a study evaluating meat quality responses in steers fed distiller's grains, it was concluded that

feeding distiller's grains at even high levels (up to 50% of diet dry matter) had no effects on tenderness or sensory attributes (Roeber et al., 2005). Likewise, another study observing the impact of diets containing distiller's grains on beef sensory attributes, determined that feeding distiller's grains had no effects on sensory traits or Warner-Bratzler shear force values of steaks (Gill et al., 2008).

History, Characteristics, and Background of Direct-fed Microbials

Probiotics or direct-fed microbials have a long and intriguing history. Probiotics have been defined as "a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance" (Fuller, 1989). Some consider the terms probiotics and direct-fed microbials interchangeable. Probiotics, however, is a generic and all-encompassing term used for microbial cultures, extracts, enzyme preparations, and is the term that is commonly used when the product is for human consumption (Elam et al., 2003). The preferred term when used in reference to products fed to livestock is direct-fed microbials. The Food and Drug Administration as well as the Association of American Feed Control Officials have required feed manufacturers to use the term "direct feed microbial" instead of probiotic in animal feeds (Miles and Bootwalla, 1989; AAFCO, 1999; FDA, 2003). Furthermore, the FDA has gone on to define direct-fed microbials as "a source of live, naturally occurring microorganisms" (Yoon and Stern, 1995; Krehbiel et al., 2003). E. Metchnikoff is considered the father of probiotics and first proposed the idea that consuming live lactobacilli capable of living inside the gastrointestinal tract was desirable (Gilliland, 1989; Yoon and Stern, 1995). Metchnikoff was searching for the always intriguing fountain of youth and studied the

life spans of people in other parts of the world. He theorized that the longevity of Bulgarian people was due to their consumption of a fermented milk product that contained lactobacilli (Gilliland, 1989; Yoon and Stern, 1995; Krehbiel et al., 2003). Metchnikoff published a book, *The Prolongation of Life*, which outlined his findings and theories in 1908. This book led to several studies on *Lactobacillus* species during the 1920's (Stern and Storrs, 1975). The early popularity of *Lactobacillus acidophilus* therapy reached its peak in the 1930's (Stern and Storrs, 1975). Following the world wars, the wide spread use and effectiveness of antibiotics that often destroyed all intestinal bacteria lead to an increase of "antibiotic diarrhea" which lead to renewed interest in *Lactobacillus acidophilus* therapy for intestinal microflora repair and restoration (Krehbiel et al., 2003). In recent years, there have been increasing societal concerns over the use of antibiotics and other growth stimulants in the livestock industry. This situation is further complicated by the increased emphasis placed on the industry to reduce diseases and pathogens while simultaneously improving production efficiency. The combination of these two things has led to an increase in interest in the effects of direct-fed microbials on animal health and performance in modern years (Krehbiel et al., 2003). The original concept of feeding a direct-fed microbial to livestock was based on the presumption of potential benefits on intestinal effects which included the establishment of more desirable microflora and the prevention of the establishment of pathogenic organisms (Krehbiel et al., 2003). Some additional responses to bacterial direct-fed microbials in cattle include: increases in average daily gains and improved feed efficiency in feedlot cattle, improved health, increased immunity, and increased performance in young calves, decreases in potential for ruminal acidosis, increases in

propionate concentrations within the rumen, and altered rumen microflora populations (Krehbiel et al., 2003; Guillen, 2009). Currently, there are at least 42 individual species of microorganisms that are approved for use in direct-fed microbials by the FDA and AAFCO (Alliance Animal Health, 2009). The two direct-fed microbial species most commonly fed to ruminants are *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* (Raeth-Knight et al., 2007). The feeding of these two organisms together is thought to be advantageous due to the individual characteristics of each organism. *Lactobacillus acidophilus* is a lactate-producing bacteria while *Propionibacterium freudenreichii* is a lactate-utilizing bacteria and produces propionate resulting from fermentation (Raeth-Knight et al., 2007).

Utilization of Direct-fed Microbials in Growing and Finishing Cattle

Society's concerns over the continued use of antibiotics in production agriculture and the increased interest in disease and pathogen prevention in the food supply have led to an increased interest in use of direct-fed microbials in growing and finishing cattle (Elam et al., 2003). Other more economical reasons for the increase in usage of direct-fed microbial products in growing and finishing cattle include improved performance, improved health responses in sick cattle, and significantly reduced mortality in heavier cattle (Krehbiel et al., 2003; McDonald et al., 2005). Cattle weighing 318 kilograms or greater (700 pounds or greater) had significantly reduced death loss when receiving a direct-fed microbial (McDonald et al., 2005). Although studies in newly received cattle or stocker cattle are limited, the results of these studies suggest that the use of a direct-fed microbial can improve the health and performance of stressed or newly received cattle

(Krehbiel et al., 2003). Feeding a single dose of a direct-fed microbial to steer calves prior to the initiation of grazing spring wheat pasture improved performance (Phillips et al., 2005). To get an idea of the extent of direct-fed microbial use in feedlots, VetLife conducted a survey (McDonald et al., 2005). Data from the VetLife Benchmark Performance Program survey in 2004 confirmed the widespread use of direct-fed microbials in feedlots (McDonald et al., 2005). The survey regarding direct-fed microbial usage in feedlots received responses from 267 feedlots and records on 10,900,504 cattle. In summation of this survey, of the 267 feedlots surveyed, 118 were using a direct-fed microbial product (McDonald et al., 2005). This amounted to over 44% of feedlots in the study that were using a direct-fed microbial product at the time of the survey. Many estimate even more widespread uses of direct-fed microbial products today.

Effects of Direct-fed Microbials on Feedlot Cattle Performance

Direct-fed microbials can impact feedlot cattle performance. In a study observing the effects of *Propionibacterium freudenreichii* and two strains of *Lactobacillus acidophilus* on feedlot steers, cattle receiving a direct-fed microbial had improved average daily gains by 6.9% (Rust et al., 2000). In the same trial, steers receiving the direct-fed microbial treatments had improved feed efficiency by 7.3% compared to those steers on the control treatment (Rust et al., 2000). McPeake et al. (2002) combined data from six research trials consisting of 1,249 head of steers to summarize the effects of *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* on feedlot performance. Contrasts were performed for direct-fed microbial steers versus control steers. These

contrasts revealed greater final live weights, overall average daily gains, and carcass adjusted average daily gains for direct-fed microbial steers (McPeake et al., 2002). Steers receiving a direct-fed microbial also tended to have greater overall dry matter intake (McPeake et al., 2002). In their review of bacterial direct-fed microbials in ruminants, Krehbiel et al. (2003) suggested that the feeding of a direct-fed microbial to feedlot cattle would result in 2.5 to 5% increase in average daily gain and a 2% improvement in feed efficiency, while dry matter intake may be inconsistent. Cattle receiving a direct-fed microbial had improved efficiency in a trial evaluating dose titration of *Lactobacillus acidophilus* combined with a single dose of *Propionibacterium freudenreichii* (Vasconcelos et al., 2008). However, feed efficiency responded quadratically with increasing doses of *Lactobacillus acidophilus* with the lower and higher *Lactobacillus acidophilus* treatments being numerically greater than the intermediate *Lactobacillus acidophilus* treatment (Vasconcelos et al., 2008). In the Vetlife survey regarding direct-fed microbial usage, it was demonstrated that cattle receiving a direct-fed microbial did exhibit improved performance (McDonald et al., 2005). Steers receiving a direct-fed microbial had 1.9% greater average daily gains and demonstrated a 1.9% improvement on feed conversion when compared to control steers (McDonald et al., 2005). Heifers on direct-fed microbials had 1.4% greater average daily gains and demonstrated a 3.9% improvement on feed conversion when compared to control heifers (McDonald et al., 2005). While there is evidence of bacterial direct-fed microbials improving performance, results have been somewhat inconsistent (Krehbiel et al., 2003). This is evidenced by another study of the effects of two strains of *Lactobacillus acidophilus* combined with a

single dose of *Propionibacterium freudenreichii* (Elam et al., 2003). Elam et al. (2003) determined that the direct-fed microbials did not affect animal performance.

Effects of Direct-fed Microbials on Carcass Traits and Carcass Merit

In addition to impacts on cattle performance, direct-fed microbials have demonstrated the potential to affect carcass characteristics. This impact is generally seen as a yield response causing increases in hot carcass weights while not affecting carcass quality (Krehbiel et al., 2003). The review of data from six research trials consisting of 1,249 head by McPeake et al. (2002) showed *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* impacted carcass characteristics. This summary confirmed that steers receiving a direct-fed microbial had greater hot carcass weights when compared to steers receiving a control diet (McPeake et al., 2002). McPeake et al. (2002) observed no significant differences in carcass quality traits for steers receiving a direct-fed microbial. Most data from direct-fed microbial research trials suggests that feeding a direct-fed microbial will not significantly impact dressing percentage, yield grade, quality grade, or any other carcass traits other than potentially increasing hot carcass weight (Elam et al., 2003; Krehbiel et al., 2003; Vasconcelos et al., 2008).

Potential Modes of Action of Direct-fed Microbials

There are several proposed modes of action for direct-fed microbials. The mode of action for a particular direct-fed microbial can vary with the type of substrate utilized, the feeding strategy employed, the forage-to-concentrate ratio of the diet, and the physiological condition or production consideration of the cattle (Wallace, 1994;

Lehloenya et al., 2008). There are certain biological conditions that must be met for a direct-fed microbial to be efficacious and have the mode of action that was intended. The direct-fed microbial should not be pathogenic, should be able to survive through all segments of the gut, should be specific to the host species, and be a stable organism (Holzapfel et al., 1998). If these biological conditions are met, it has been suggested that direct-fed microbials are able to: produce organic acids, competitively exclude potentially harmful bacteria, stimulate immune system responses, produce antibiotics, produce enzymes and increase enzyme activity, and reduce toxic amines (Krehbiel et al., 2003; Alliance Animal Health, 2009).

Through the production of organic acids, specifically lactic, acetic, and formic acids, direct-fed microbials can inhibit intestinal pathogens or serve as an energy source to other beneficial bacteria and ultimately the animal (Krehbiel et al., 2003; Alliance Animal Health, 2009). It has also been suggested that direct-fed microbials can competitively exclude other bacteria present in the gut. That is, direct-fed microbials could compete with pathogenic bacteria for attachment sites in the intestines and could in turn reduce pathogen loads in the intestine (Salimen et al., 1996; Krehbiel et al., 2003).

Direct-fed microbials can stimulate immune system responses. Bacterial direct-fed microbials have demonstrated effects on the innate, humoral, and cellular elements of the immune system (Krehbiel et al., 2003). In addition to the gastrointestinal tract's roles in digestion and absorption of nutrients, it also provides a line of defense against the constant presence of antigens in the gut from food and harmful microorganisms (Krehbiel et al., 2003). Certain strains of bacteria have actual antimicrobial properties. Many species of lactobacilli have been shown to inhibit pathogens (Krehbiel et al., 2003).

Lactobacilli have also been shown to produce hydrogen peroxide which demonstrates bactericidal activity (Krehbiel et al., 2003).

Direct-fed microbials can also affect enzyme activity within the host animal. Beneficial *Bacillus* spp. bacteria produce a wide variety of enzymes including proteases, amylases, lipases, and glycosidases (Alliance Animal Health, 2009). Direct-fed microbials can additionally cause reductions in toxic enzymes within the intestines. Amines produced by some microbes are toxic and have been associated with diarrhea (Alliance Animal Health, 2009). Lactic acid bacteria can reduce amine concentrations and neutralize enterotoxins within the gut (Alliance Animal Health, 2009).

In addition to these general modes of action, there are targeted modes of actions for different types of direct-fed microbials or combinations of direct-fed microbials. The most well documented example would be utilizing a lactate-producing bacteria such as *Lactobacillus acidophilus* in combination with a lactate-utilizing bacteria such as *Propionibacterium freudenreichii* (Raeth-Knight et al., 2007). In this particular example, the presence of the lactate-producing *Lactobacillus acidophilus* is helping the ruminal microorganisms adapt to the presence of lactic acid (Ghorbani et al., 2002; Beauchemin et al., 2003). The presence of the lactate-utilizing *Propionibacterium freudenreichii* is helping to prevent lactate from accumulating in the rumen (Kung and Hession, 1995; Beauchemin et al., 2003). The intended result of this example would be a decrease in the risk of acidosis and improved feed digestion in feedlot cattle receiving a high-grain diet (Beauchemin et al., 2003).

Escherichia coli O157:H7 Characteristics

Escherichia coli is a facultative anaerobic bacterium that is commonly found in the intestinal tract of mammals, especially ruminant animals, which are reservoirs for the pathogen (Callaway et al., 2009). *E. coli* subsists by fecal-oral means, and can comprise up to 1% of the gastrointestinal tract bacterial population (Callaway et al., 2009). *E. coli* O157:H7 has received much attention because of its connection with food borne illness (Loneragan and Brashears, 2005; Guillen, 2009). *E. coli* serotype 0157 has been well characterized as a food borne pathogen to humans due to several factors that contribute to health risks from exposure to the pathogen (Mead et al., 1999; LeBlanc, 2003; Callaway et al., 2009; Guillen, 2009). These factors include the expression of intimin, which is required for attachment to the host cell and the formation of attachment lesions, the production of Shiga toxins, which are key virulence factors and act to inhibit protein synthesis within target cells, and the production of enterohemolysins, which are plasmid-encoded toxins that can readily cause the hemolysis of erythrocytes (LeBlanc, 2003; Kaper et al., 2004; Dean-Nystrom et al., 1998; Loneragan and Brashears, 2005; Guillen, 2009). The disease caused by *E. coli* O157:H7 is characterized by hemorrhagic colitis which can lead to bloody diarrhea, non-bloody diarrhea, and hemolytic uremic syndrome (Guillen, 2009). Strains of *E. coli* that cause diarrhea are referred to as enterohemorrhagic. The most enterohemorrhagic serotype to humans in the United States is O157:H7 (Guillen, 2009). Each year in the U.S. more than 60 people die and 73,000 are made ill by *E. coli* O157:H7 and entrohemorrhagic *E. coli* infections are estimated to cost the U.S. economy more than $1,000,000,000 (Mead et al., 1999; USDA, ERS, 2001; Callaway et al., 2009). The major concern with this pathogen is preventing *E. coli*

outbreaks, such as the first known outbreak which was associated with hamburgers in 1982 (Riley et al., 1983; Guillen, 2009). Ground beef is most frequently blamed as the source of *E. coli* outbreaks (Callaway et al., 2009). Multiple large-scale ground beef recalls due to *E. coli* O157:H7 contamination and the well-publicized deaths of children who consumed foods contaminated by *E. coli* linked to beef products have hurt consumer confidence to the wholesomeness and safety of beef (Gage, 2001; Callaway et al., 2009). While disease attributed to *E. coli* infections can occur as outbreaks, most of the cases of *E. coli* are sporadic and not associated with an outbreak event (Loneragan and Brashears, 2005). In addition, reported cases of *E. coli* O157:H7 contamination in ground beef have declined in recent years, and this decline in contamination is concurrent with a decrease in reported *E. coli* infections (Loneragan and Brashears, 2005; LeJeune and Wetzel, 2007).

Prevalence of *Escherichia coli O157:H7* in Cattle

Cattle are considered to be the primary reservoir for the pathogen *Escherichia coli* O157:H7 (Greenquist et al., 2005; Loneragan and Brashears, 2005; Callaway et al., 2009; Guillen, 2009). Recent studies using molecular and immunomagnetic techniques have led to more accurate estimations of *E. coli* shedding in cattle. It is estimated that approximately 30% of feedlot cattle are carriers of *E. coli* O157:H7, with the highest incidence of *E. coli* shedding by cattle taking place in the summer months (Greenquist et al., 2005; Loneragan and Brashears, 2005; Callaway et al., 2009). According to numerous large-scale research studies, *E. coli* O157:H7 prevalence is widespread throughout feedlots and the entire cattle population (Loneragan and Brashears, 2005;

LeJeune and Wetzel, 2007; Guillen, 2009). Although the presence of *E. coli* O157:H7 in a given feedlot is almost certain, observed prevalence greatly varies pen-to-pen while it may not vary substantially from feedlot-to-feedlot (Loneragan and Brashears, 2005; LeJeune and Wetzel, 2007; Guillen, 2009).

Escherichia coli can rarely be cultured from the rumen of cattle in high numbers (Callaway et al., 2009). *E. coli* is rarely present at more than 10^6 cells per milliliter, out of a total population that is greater than 10^{10} cells per milliliter (Laven et al., 2003; Callaway et al., 2009). Conditions are much more favorable for *E. coli* in the lower tract. Colonization by *E. coli* O157:H7 occurs in the lower gastrointestinal tract, specifically on the mucosal surface of the rectum (Naylor et al., 2003; Jacob et al., 2008; Callaway et al., 2009). In the lower tract, *E. coli* concentrations can range from 10^2 to 10^7 cells per gram of feces (Jordan and McEwen, 1998; Callaway et al., 2009). Following colonization the organism is spread by shedding through the feces (Naylor et al., 2003; Jacob et al., 2008; Callaway et al., 2009). Contrary to common thought, hide prevalence of *E. coli* appears to be the major source of carcass contamination and a more accurate predictor of carcass contamination than fecal prevalence of *E. coli* (Loneragan and Brashears, 2005; Callaway et al., 2009). In all likelihood, however the hide contamination is a result of *E. coli* being present in the feces (Loneragan and Brashears, 2005). The cattle industry has invested huge sums of money and resources while both pubic and private researchers have devoted much time and energy toward improving the safety of meat products at the time of harvest and processing (LeJeune and Wetzel, 2007). Some of the significant results of this effort include the implementation of hazard analysis and critical control point policies and enhanced post-slaughter sanitation methods which have resulted in a

decrease in the frequency that ground beef is contaminated with *E. coli* O157:H7 (CDC, 2005; LeJeune and Wetzel, 2007).

Incidence of *Escherichia coli O157:H7* in Cattle Fed Wet Distiller's Grains plus Solubles

While wet distiller's grains plus solubles has demonstrated greater energy values and improved cattle performance compared to corn, there are some concerns with feeding wet distiller's grains, especially at high inclusion levels (Vander Pol et al., 2006b; Klopfenstein et al., 2008). One such concern is that some research has indicated that there is a connection between feeding distiller's grains and increased *E. coli* shedding in feedlot cattle (Jacob et al., 2008). While there had been a decline in *E. coli* incidents in recent years there was a substantial increase in 2007. In 2007, there were 20 ground beef recalls due to *E. coli* O157:H7 compared to only eight in 2006 (Klopfenstein et al., 2009). A number of people theorized this was due to rapid growth of the ethanol industry in 2007 and the simultaneous increase in feeding of ethanol byproducts including wet distiller's grains plus solubles (Klopfenstein et al., 2009). Jacob et al. (2008) observed that cattle fed a diet containing 25% dried distiller's grains had higher ($P = 0.01$) prevalence of *E. coli* O157:H7 in fecal samples compared to cattle fed a diet with no dried distiller's grains. In addition, ruminal microbial fermentations were performed and steers fed a diet containing dried distiller's grains had greater *E. coli* O157:H7 prevalence in these fermentations than steers fed no dried distiller's grains (Jacob et al., 2008). These results led Jacob et al. (2008) to conclude the there was a positive association between distiller's grains and *E. coli* O157:H7 prevalence in cattle. In a study utilizing manure slurries from cattle fed 0, 20, 40, or 60% wet distiller's grains plus solubles,

Varel et al. (2008) concluded that feeding wet distiller's grains plus solubles could extend the persistence of *E. coli* O157:H7. Another trial investigating the prevalence of *E. coli* O157:H7 in feces and on hides of feedlot steers, showed the animals fed 40% wet distiller's grains plus solubles in the diet had a higher prevalence of *E. coli* O157:H7 in the feces and on hides (Wells et al., 2009). This study took place from October through June and the authors determined that feeding wet distiller's grains plus solubles could increase both the level and prevalence of *E. coli* O157:H7 when *E. coli* would normally be seasonally low (Wells et al., 2009). Recently, Jacob et al. (2009) conducted a larger study that evaluated *E. coli* O157:H7 prevalence in cattle fed distiller's grains consisting 700 cattle where 3,560 samples were collected and analyzed. While *E. coli* O157:H7 prevalence was numerically higher in cattle fed distiller's grains for certain weeks, there was not an overall significant effect of distiller's grain inclusion on *E. coli* O157:H7 prevalence (Jacob et al., 2009). Results of research involving distiller's grains and *E. coli* O157:H7 have been inconsistent, and most studies showing a positive association were feeding distiller's grains at levels greater than what is being feed in commercial feedlots (Klopfenstein et al., 2009). Currently, there is no consistent evidence that feeding distiller's grains at the levels being used commercially increases *E. coli* shedding (Klopfenstein et al., 2009).

Pre-harvest Control of *Escherichia coli O157:H7* in Cattle

Since cattle are the primary reservoir for *E. coli* O157:H7, and beef products are repeatedly linked to cases of *E. coli* O157:H7 in humans, it would be extremely advantageous to decrease the prevalence and magnitude of *E. coli* O157:H7 shedding in

cattle prior to harvest (Greenquist et al., 2005; Loneragan and Brashears, 2005; LeJeune and Wetzel, 2007; Callaway et al., 2009; Guillen, 2009). There are three broad categories into which *E. coli* O157:H7 preharvest intervention practices can be grouped (LeJeune and Wetzel, 2007). Preharvest intervention methods include exposure reduction, exclusion, and direct antipathogen strategies (LeJeune and Wetzel, 2007). Some strategies for decreasing exposure in cattle include monitoring water quality and preventing water contamination, monitoring feed hygiene and feed components, minimizing environmental exposure and risk factors, maintaining proper animal density in pens, and excluding wildlife from water, feed, and pens (Loneragan and Brashears, 2005; LeJeune and Wetzel, 2007; Callaway et al., 2009; Guillen, 2009). Preharvest *E. coli* exclusion practices include feed and ration ingredient and management strategies and the utilization of probiotics, or direct-fed microbials, and prebiotics that are unavailable to or undigested by cattle, but available to specific bacteria (Loneragan and Brashears, 2005; LeJeune and Wetzel, 2007; Callaway et al., 2009; Guillen, 2009). Finally, there are direct antipathogen strategies which include hide washing, utilization of antimicrobial compounds, such as neomycin sulfate and sodium chlorate, bacteriophage therapy, and vaccination (Loneragan and Brashears, 2005; LeJeune and Wetzel, 2007; Callaway et al., 2009; Guillen, 2009). Many of these strategies are impractical or not readily practiced for various reasons. In addition, most of these strategies have yielded inconsistent results (Callaway et al., 2009). Currently, there is only one method of preharvest control for *E. coli* O157:H7 in cattle that has been both effective and gained widespread acceptance by the cattle industry. This method is feeding a *Lactobacillus*-based direct-fed microbial to cattle prior to harvest (Loneragan and Brashears, 2005; LeJeune and Wetzel, 2007).

The Use of Direct-fed Microbials to Control the Shedding of *Escherichia coli O157:H7*

The use of direct-fed microbials, specifically *Lactobacillus*-based direct-fed microbials, to control the shedding of *E. coli* O157:H7 in cattle has received much consideration from both researchers and the cattle industry (Loneragan and Brashears, 2005; LeJeune and Wetzel, 2007). *Lactobacillus*-based direct-fed microbials have repeatedly demonstrated effectiveness in decreasing *E. coli* O157:H7 shedding in cattle (Loneragan and Brashears, 2005; LeJeune and Wetzel, 2007). In a study evaluating *E. coli* O157:H7 prevalence in feedlot cattle by Brashears et al. (2003), it was discovered that the feeding of *Lactobacillus acidophilus* NPC 747 decreased *E. coli* O157:H7 shedding in the feces of cattle when compared to the control diet. In addition, supplementation with a direct-fed microbial decreased the incidence of *E. coli* O157:H7 in the pens and the number of *E. coli* O157:H7 positive hides at harvest (Brashears et al., 2003). These results led Brashears et al. (2003) to suggest that the feeding of *Lactobacillus*-based direct-fed microbial would decrease fecal shedding of *E. coli* O157:H7 and contamination on hides. Another trial observed *E. coli* O157:H7 prevalence with various levels of *Lactobacillus acidophilus* NP51 in combination with *Propionibacterium freudenreichii* (Younts-Dahl et al., 2005). Cattle receiving *Lactobacillus acidophilus* in combination with *Propionibacterium freudenreichii* had a lower prevalence of *E. coli* O157:H7 throughout the feeding period, and there was a linear decrease in prevalence with increasing dose of *Lactobacillus acidophilus* (Younts-Dahl et al., 2005). These results led Younts-Dahl et al. (2005) to conclude that the feeding of *Lactobacillus acidophilus* NP51 was an effective preharvest *E. coli* intervention strategy. In another study, steers were given different strains of

Lactobacillus acidophilus to evaluate the prevalence and enumeration *E. coli* O157:H7 in cattle fed a direct-fed microbial (Stephens et al., 2007). The prevalence of *E. coli* O157:H7 in control cattle was greater ($P < 0.05$) than in cattle receiving *Lactobacillus acidophilus* strains NP51, NP28, or NP51-NP35 (Stephens et al., 2007). Tabe et al. (2008) observed that steers receiving a *Lactobacillus acidophilus* direct-fed microbial had a significant reduction in fecal shedding of *E. coli* O157:H7 when compared to control steers during the finishing period. The steers on the *Lactobacillus acidophilus* treatment had a 32% decrease in the fecal shedding of *E. coli* O157:H7 (Tabe et al., 2008). While the feeding of direct-fed microbials have shown inconsistent results, these studies indicate that direct-fed microbials have the ability to decrease the shedding of *E. coli* O157:H7 in cattle.

Summary and Conclusions from the Literature

Food safety is a major concern to everyone involved the agriculture industry, especially in production animal agriculture. An extremely important part of food safety is the reduction of human pathogens which can lead to foodborne illness. The pathogen that has received the most attention in recent years is *Escherichia coli* O157:H7. Since cattle are the main reservoir for *E. coli* and beef is most commonly implicated in *E. coli* O157:H7 infections, pathogen control should be a concern of cattle producers as well as those in the food industry. Feedlot managers should be conscious of feedstuffs which potentially increase *E. coli* O157:H7 shedding as well as methods to reduce this pathogen in the live animal.

As long as ethanol and biofuel production remains constant or continues to increase, ethanol by-products will continue to be an important feedstuff in the formulation of cattle diets. Feedlot managers must find ways to effectively utilize wet distiller's grains in feedlot diets to be able to formulate least cost rations as well as capitalize on the greater energy value and improved cattle performance distiller's grains offer compared to corn. While there are several advantages to feeding wet distiller's grains, cattle feeders must be mindful of concerns with feeding wet distiller's grains plus solubles, notably the potential to increase incidence of *Escherichia coli* O157:H7 shedding in cattle.

One potential way of combating *E. coli* O157:H7 shedding in feedlot cattle would be through the feeding of direct-fed microbials. Direct-fed microbials have received much consideration from the feedlot industry in recent years due to the perception that there is a need for disease prevention and enhanced performance while at the same time reducing the industry's dependence on antimicrobial use in beef production. Direct-fed microbials the potential to improve production efficiency in feedlot cattle, alter ruminal fermentation, and decrease the shedding of *E. coli*.

CHAPTER III

FEEDING A DIRECT-FED MICROBIAL TO DETERMINE PERFORMANCE, CARCASS CHARACTERISTICS, AND FECAL SHEDDING OF *ESCHERICHIA COLI* O157:H7 IN FEEDLOT HEIFERS FED WITH OR WITHOUT WET DISTILLER'S GRAINS PLUS SOLUBLES

Abstract

Fluctuating corn prices related to increased ethanol production have had a significant impact on the cost of gain for cattle feeders that rely on corn-based diets. The inclusion of wet distiller's grains plus solubles (WDGS) in feedlot diets has become a common practice in many regions of the U.S. due to the expanded production of co-products. In addition, societal concerns over the continued use of antimicrobials in production animal agriculture combined with an enhanced interest in disease and pathogen prevention in the food supply have led to an increased interest in use of direct-fed microbials (DFM) in growing and finishing cattle. Direct-fed microbials have been shown to improve ADG and feed efficiency, alter ruminal fermentation, and decrease fecal shedding of potential harmful pathogens in feedlot cattle. The objective of this experiment was to evaluate the effects of *Lactobacillus acidophilus* combined with *Propionibacterium freudenreichii* on performance, carcass characteristics, and *Escherichia coli* O157:H7 shedding in feedlot heifers fed with or without WDGS.

Crossbred heifers (n = 288; initial BW = 295 ± 28 kg) were assigned to 1 of 4 treatments (12 pens per treatment; 6 heifers per pen) in a randomized complete block design with a 2 × 2 factorial arrangement of treatments. Across the feeding period, heifers fed 30% WDGS tended ($P = 0.09$) to have greater ADG and had greater carcass-adjusted ADG ($P = 0.05$) compared with heifers fed dry-rolled corn (DRC). Dry matter intake was not affected ($P = 0.65$) by diet, although carcass adjusted G:F tended ($P = 0.10$) to be improved for heifers fed WDGS. Heifers fed 30% WDGS tended ($P \leq 0.10$) to have greater fat thickness at the 12th rib, lower marbling scores, and higher yield grades. The inclusion of *L. acidophilus* combined with *P. freudenreichii* in the diet had no effect ($P > 0.10$) on performance or carcass merit in the present experiment. The incidence of *Escherichia coli* O157:H7 throughout the experiment was low, with only 18 positive samples across all sampling periods. Neither WDGS inclusion nor the inclusion of *L. acidophilus* combined with *P. freudenreichii* in the diet had any effect ($P > 0.10$) on *E. coli* O157:H7 shedding in this experiment. Feeding 30% WDGS to feedlot heifers improved animal performance compared to the DRC based control diet.

Key Words**:** beef cattle, direct-fed microbials, wet distiller's grains plus solubles, *Lactobacillus acidophilus, Propionibacterium freudenreichii*

Introduction

The expanded production of ethanol in recent years has caused an increase in the production of co-products. Co-products, especially wet distiller's grains plus solubles (WDGS), can be utilized very efficiently by ruminants (Klopfenstein et al., 2008). The

increased ethanol production has also contributed to fluctuating corn prices which impact cattle feeders that rely on corn-based diets. These factors have caused the inclusion of WDGS in feedlot diets to become a common practice. There are numerous benefits associated with the feeding of WDGS to feedlot cattle, including improved cattle performance (Klopfenstein et al., 2008). However some research has indicated there is a connection between feeding distiller's grains and increased *Escherichia coli* shedding in feedlot cattle (Jacob et al., 2008).

Current public perception is that there is a need for sufficient disease and pathogen prevention while simultaneously enhancing performance and reducing antimicrobial use in feedlots. As a result, direct-fed microbials (DFM) have received much consideration as they are a source of live, naturally occurring microorganisms (Yoon and Stern, 1995). In a review of DFM utilization consisting of 10,900,504 cattle in 73,870 feedyards, steers and heifers had 1.9% and 1.4% improved ADG, respectively, when receiving a DFM (McDonald et al., 2005). Additionally, studies have shown that feeding a DFM may reduce the fecal shedding of *E. coli* O157:H7 (Elam et al., 2003; Peterson et al., 2007). Data suggest that DFM have the potential to improve production efficiency in cattle and decrease the shedding of potential harmful pathogens, including pathogens that could be transmitted to humans. The objective of this study was to evaluate the effects of *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* on performance, carcass characteristics, and *Escherichia coli* O157:H7 shedding of feedlot heifers fed a high-concentrate diet with or without WDGS.

Materials and Methods

Experimental Design and Animals

Two hundred and eighty-eight crossbred heifers (BW at arrival = 295 ± 28 kg) were delivered to the Willard Sparks Beef Research Center at Oklahoma State University. On arrival at the feed yard, heifers were individually weighed and a uniquely numbered ear tag was placed in the left ear of each calf. On the morning following arrival, heifers were individually weighed, vaccinated for protection against infectious bovine herpes virus-1, bovine viral diarrhea virus (types I and II), bovine parainfluenza-3, and bovine respiratory syncytial virus (Vista 5 SQ; Intervet, Millsboro, DE), *Clostridium chauvoei, septicum, novyi, sordellii,* and *perfringens* Types C and D (Vision 7 with SPUR, Intervet, Millsboro, DE), treated for control of external and internal parasites (Ivomec-Plus injectable; Merial, Duluth, GA), and implanted with Revalor IH (Intervet, Millsboro, DE). Initial body weights were obtained by using the average BW of the heifers on consecutive days. The heifers were then blocked by initial BW into 12 weight blocks. Within block, heifers were randomly assigned to 4 pens (12 pens per treatment; 6 heifers per pen). Heifers were reimplanted based on BW with Revalor H (Intervet, Millsboro, DE) on day 56 (6 heaviest weight blocks) or day 84 (6 lightest weight blocks).

Treatments and Diets

Heifers were assigned to 1 of 4 treatments in a randomized complete block design with a 2 × 2 factorial arrangement of treatments. Heifers were assigned to either a diet containing 30% wet distiller's grains plus solubles (WDGS) or a dry-rolled corn (DRC) based control diet. The DRC based control diet was formulated with additional fat in an

attempt to formulate diets that were isocaloric. The WDGS utilized in this experiment was purchased and shipped to the feedlot from East Kansas Agri-Energy, Garnett, KS. Within the dietary treatments, heifers were assigned to a direct-fed microbial (DFM) treatment that was color-coded and blinded to research personnel until the conclusion of the study. The DFM product utilized was a commercially available DFM containing *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* (Bovamine Rumen Culture; Nutrition Physiology Company, LLC, Guymon, OK). The treatments consisted of the DFM, containing 1×10^6 colony forming units of *Lactobacillus acidophilus* combined with 1×10^9 colony forming units of *Propionibacterium freudenreichii* or the control treatment containing no DFM. The diets were fed from day 1 through finish. Cattle were fed ad libitum twice daily at 0600 hours and 1300 hours. The WDGS finishing diet contained 58.0% DRC and 30.0% WDGS, and was formulated to meet or exceed NRC (1996) nutrient requirements (Table 1). The DRC finishing diet contained 80.75% DRC, and was formulated to meet or exceed NRC (1996) nutrient requirements (Table 1). Monensin (Rumensin; Elanco, Greenfield, IN) was fed at a rate of 33 mg/kg of diet. Tylosin (Tylan; Elanco, Greenfield, IN) was fed at a rate of 10 mg/kg of diet. Heifers were gradually adapted to their final treatment diet using 3 step-up diets shown in Table 1. Step-up diets were fed for seven days each. Experimental treatments were provided via a dry ground corn premix containing the experimental cultures and fed at the rate of 227 g (0.50 lb) per head daily top dressed onto the total mixed ration and mixed in the complete diet in each individual pen's feed bunk. Control treatments received equal amounts of the dry ground corn premix containing no DFM fed at the same rate per head daily top dressed onto the total mixed ration and mixed in the complete diet in each

individual pen's feed bunk. Prior to mixing, the DFM and the control (equal amount of ground corn containing no DFM) were stored in a freezer in color-coded individual packets. The individual premixes for each DFM treatment were initially mixed with 1,814 g (4 lb) of ground corn using 2 separate KitchenAid mixers (5 QT Artisan Mixer Model 5SM150PS, KitchenAid St. Joseph, MI). This premix was divided in half 907 g (2lbs) and then mixed with 15.4 kg (34 lb) of ground corn in 2 separate cement mixers (Red Lion Big Cat, Monarch Industries, Winnipeg, Manitoba, Canada). This was repeated with the second half of the initial premix and 15.4 kg (34 lb) of ground corn yielding a total of 16.3 kg of total premix per treatment (36 lb total/treatment). Mixers were dedicated to each individual DFM treatment throughout the experiment in order to prevent any cross contamination of treatments. One thousand three hundred and sixty-one grams (3 lb) of the premix were then weighed into individual 3.8 L (1 gallon) color-coded plastic containers assigned to the appropriate treatment pen. Contents of the appropriate container were mixed directly into the feed after feed was delivered to the bunk in pens of cattle assigned to that treatment. Feed refused was weighed on each weigh day and as needed (e.g., following inclement weather) for DM determination. In addition, diet samples were collected, and DM content of diets and dietary ingredients determined. Diet samples were dried in a forced-air oven (60ºC) and ground in a Wiley mill to pass a 1-mm screen. Diet samples were analyzed for ash, N, starch (AOAC, 1990), NDF, ADF (Goering and Van Soest, 1970), and Ca, P, and K (Table 1).

Body Weights

Interim unshrunk BW was determined by weighing pens and individual animals on days 28, 56, 84, 119, and immediately prior to shipping for harvest (shipped in 3 separate groups). Pen weights were used for statistical analysis as pen was the experimental unit. For calculating ADG, weights taken on all days were shrunk 4%. The heaviest pens (8 pens) were harvested after 132 days on feed, the medium weight pens (20 pens) were harvested after 167 days on feed, and the lightest weight pens (20 pens) were harvested after 188 days on feed. Carcass adjusted BW was calculated by taking the HCW divided by the average dressing percentage for each of 3 harvest groups (light, medium, and heavy). Carcass adjusted BW was then used to calculate carcass adjusted ADG and carcass adjusted G:F.

Carcass Data and Liver Scores

The heifers were harvested at Cargill Meat Solutions, Dodge City, KS. The heifers were shipped to be harvested in 3 separate groups (light, medium, and heavy). Trained personnel from Oklahoma State University along with Cargill personnel obtained all carcass measurements. Measurements included hot carcass weight, liver abscess score (data collected by Cargill personnel), longissimus muscle area and marbling score of the split lean surface at the 12th/13th rib interface, percentage of kidney, pelvic, and heart (KPH) fat, fat thickness at the ¾ measure opposite the split lean surface between the 12th and 13th rib, USDA Yield Grade, and USDA Quality Grade. Liver abscess scores were recorded on a scale of 0 to 6, with 0 = no abscesses, 1 = A-, 2 = A, 3 = A+, 4 =

telangiectasis, 5 = distoma (fluke damage), and 6 = fecal contamination that occurred at slaughter.

Escherichia coli Shedding

Fecal samples obtained from each animal per rectum on all weighdays were kneaded and approximately 1 g of fecal material was placed in 9 mL of Gram Negative (GN) broth supplemented with cefixime (0.05 mg/L), cefsulodin (10.0 mg/L), and vancomycin (8.0 mg/L; GNccv). Samples were vortexed for 1 min and incubated for 5 h at 37°C. Immunomagnetic separation (IMS; Dynal, Inc.) was performed following enrichment, and 50 µL of product was plated onto sorbitol MacConkey agar supplemented with cefixime (50 ng/mL) and potassium tellurite (2.5µg/mL; CT-SMAC). Plates were incubated overnight at 37°C and up to six sorbitol negative colonies from each sample were picked and streaked onto blood agar plates. Blood agar plates were incubated overnight at 37°C and colonies were tested for indole production, the presence of the O157 antigen using latex agglutination, and confirmation of species with PCR analysis of *eae*, *fliC*, *stx*1, *stx*2, *hylA*, and *rfb*E virulence genes. A semi-quantitative method was employed to categorize fecal culture positive cattle into low shedders ($< 5 \times 10^4$ CFU/g) and high shedders ($> 5 \times 10^4$ CFU/g) (Sanderson et al., 2007). Briefly, a swab of 1:10 diluted fecal suspension in GNccv broth before enrichment was plated onto a CT-SMAC plate and incubated for 16 to 18 h at 37°C. From direct streaked CT-SMAC plates, up to six sorbitol negative colonies were transferred to a blood agar plate and evaluated for indole production, latex agglutination for the O157:H7 antigen, and PCR. This direct streaking of pre-enriched fecal sample identifies samples with *E. coli*

O157:H7 concentrations > 10^3 CFU/g with sensitivity and specificity estimates of 83% and 92%, respectively (Sanderson et al., 2007).

Calculations and Statistical Analysis

Data for BW, ADG, DMI, gain efficiency (G:F), and parametric carcass characteristics were analyzed as a randomized complete block design using the PROC MIXED procedure of SAS Release 9.1.3 (SAS Institute Inc., Cary, NC). Non-parametric USDA Quality Grade data was transformed using the Freedman's test by listing the percentage of Choice and Select for each pen within a block, and then analyzed as the normally distributed data as above. Pen was the experimental unit. The model statement included treatment, and the random statement included block. For the *E. coli* shedding data, initially the data were modeled in the GLIMMIX procedure of SAS with collection day, diet, and DFM included as fixed effects. Pen was included as a random effect. Samples that were missing or duplicate sample numbers on a collection day were included as missing values in the data set. Two animals that only had one observation were removed from the data set entirely. Analysis could not be completed on these models, likely because of low prevalence. Therefore, the FREQ procedure of SAS was used to run a chi-square analysis of data (ignoring pen and collection day) with diet and DFM as categories.

Results and Discussion

Heifer Performance

Feedlot performance data from across the feeding period is presented in Tables 2 and 3. Two interactions were observed during the first 28 days of the experiment. There was a WDGS × DFM interaction for both ADG ($P = 0.01$; Table 2) and G:F ($P = 0.04$; Table 3) from days 1 to 28. Average daily gain was greater for heifers fed the 30% WDGS diet without the DFM and the DRC diet with the DFM compared to the 30% WDGS diet with the DFM and the DRC diet without the DFM from days 1 to 28 (Table 2; Figure 1). The same trend was observed in G:F from days 1 to 28 with the 30% WDGS diet without the DFM and the DRC diet with the DFM having improved G:F compared to the 30% WDGS diet with the DFM and the DRC diet without the DFM (Table 3; Figure 2). No other interactions were observed throughout the experiment.

Heifers receiving 30% WDGS in their diet had numerically improved performance compared to heifers receiving the DRC control diet. The BW of heifers receiving 30% WDGS tended ($P = 0.06$) to be heavier on day 84 compared with heifers receiving the DRC control diet. Final BW was not different for heifers fed 30% WDGS compared to heifers receiving the DRC control diet. However heifers fed 30% WDGS had 1.7% higher average final BW ($P = 0.14$). In addition, heifers fed the 30% WDGS tended ($P = 0.08$) to have greater ADG and had greater carcass-adjusted ADG ($P = 0.05$) compared with heifers fed DRC. Gain:Feed was not significant ($P = 0.19$), but was numerically improved for heifers fed WDGS. Carcass adjusted G:F also tended ($P = 0.10$) to be improved for heifers fed WDGS. We calculated the feeding value of the WDGS in the diet as described by Klopfenstein et al. (2008). This resulted in a feeding

value of 110% for the WDGS compared to the DRC. Average DMI was not affected (P = 0.65) by diet, although heifers fed the 30% WDGS had greater DMI (P = 0.01) from days 29 to 56.

The improved performance for heifers receiving WDGS are consistent with previous research. It is well established that WDGS can improve cattle performance when compared to corn based control diets (Klopfenstein et al., 2008). Wet distiller's grains plus solubles based diets have been shown to have greater feeding values and improved G:F when compared to corn based control diets (Vander Pol et al., 2006b; Klopfenstein et al., 2008). Klopfenstein et al. (2008) reported the feeding values for WDGS between 126% and 145% of the feeding value of corn. These feeding values are higher than the calculated feeding value from the present experiment. However in the present experiment, diets were formulated to be isocaloric where added fat was included in the DRC based control diet. Many of the experiments with feeding values for WDGS included in the meta analysis by Klopfenstein et al. (2008) did not attempt to formulate diets that were isocaloric. This should be considered when evaluating the feeding value of WDGS in diets as distiller's grains contain a greater percentage of fat than ingredients being replaced in the diet. To get an accurate feeding value comparison, the diets should be balanced for fat content to avoid large differences in the energy content of diets being compared. This method results in reduced feeding values for diets containing WDGS and a more realistic comparison to corn-based diets. May et al. (2010) conducted an experiment where both corn and sorghum WDGS were fed in steam-flaked corn based diets. Additional fat was added to the diets in an attempt to formulate diets that were isocaloric (May et al., 2010). Final BW, ADG, and carcass adjusted G:F were less for

cattle fed WDGS compared to cattle fed the control diet (May et al., 2010). No differences were observed in calculated NE_m and NE_g values for the average of diets containing WDGS compared to the steam-flaked corn control diet (May et al., 2010). However, cattle fed corn WDGS or a blend of corn and sorghum WDGS had greater calculated NE_m and NE_g values compared to cattle fed only sorghum WDGS (May et al., 2010). These studies emphasize the importance of balancing diets for fat content when evaluating the energy value of dietary ingredients.

Research has demonstrated that increasing WDGS quadratically affects ADG and DMI with both ADG and DMI being maximized at 20 to 30% of the diet on a DM basis (Klopfenstein et al., 2008). In diets containing WDGS, G:F tends to be more linear and is maximized at higher inclusion levels, up to 30 to 50% of diet DM (Klopfenstein et al., 2008). The meta-analysis suggests that the optimum level of wet distiller's grains to include in diets to maximize cattle performance lies somewhere between 20 and 30% for DRC based diets (Klopfenstein et al., 2008).

In the present experiment, the inclusion of the DFM product did not improve animal performance. In a Vetlife survey of in 267 feedlots with records on 10,900,504 cattle, it was demonstrated that cattle receiving a DFM exhibited improved performance (McDonald et al., 2005). Steers receiving a DFM had 1.9% greater ADG and a 1.9% improvement on feed conversion when compared to control steers (McDonald et al., 2005). Heifers fed a DFM had 1.4% greater ADG and a 3.9% improvement on feed conversion compared to control heifers (McDonald et al., 2005). While there is evidence that bacterial DFM improve performance, results have been inconsistent (Krehbiel et al., 2003). This is evidenced by another study of the effects of two strains of *Lactobacillus*

acidophilus combined with a single dose of *Propionibacterium freudenreichii* in which Elam et al. (2003) determined that the DFM did not affect animal performance.

Carcass Characteristics

The carcass merit data is presented in Table 4. There were no differences ($P >$ 0.10) among treatments for HCW, dressing percentage, longissimus muscle area, KPH, USDA Quality Grade, or liver abscess score. However, heifers fed 30% WDGS tended to have greater fat thickness at the 12th rib, lower marbling scores, and higher yield grades ($P = 0.10$, $P = 0.09$, and $P = 0.07$, respectively). These results are consistent with previous research which suggests there are undesirable changes in carcass composition in cattle fed diets with high levels of WDGS (Reinhardt et al., 2007; Klopfenstein et al., 2008). Klopfenstein et al. (2008) demonstrated that 12th rib fat thickness and yield grade responded quadratically to increasing WDGS in the diet. In an additional meta-analysis, Reinhardt et al. (2007) showed that diets containing low levels of distiller's grains (16% and lower) increased marbling score, while diets containing high levels of distiller's grains (33% and higher) decreased marbling score. Corrigan et al. (2009) suggested that in DRC diets the inclusion of up to 27.5% WDGS increased marbling score which contradicts what we observed in this study. Impacts of WDGS on carcass merit and characteristics have demonstrated mixed results.

The inclusion of *L. acidophilus* combined with *P. freudenreichii* in the diet had no significant effect ($P > 0.10$) on carcass merit in the present experiment. However, heifers that received the combination of *L. acidophilus* combined with *P. freudenreichii* had numerically higher (2.4%) average marbling scores ($P = 0.33$) than those heifers

receiving no DFM. Most data from DFM research trials suggests that feeding a DFM will not significantly impact dressing percentage, yield grade, quality grade, or any other carcass traits, with the exception of potentially increasing hot carcass weight (McPeake et al., 2002; Krehbiel et al., 2003; Vasconcelos et al., 2008).

Escherichia coli Shedding

Results for the *E. coli* shedding data were unable to be sufficiently evaluated due to the low prevalence of *E. coli* O157:H7 throughout the entire study. *Escherichia coli* was observed in only 1.2% (18 of 1,415 samples) of the fecal samples. This was potentially due to the trial taking place in the fall and winter. *Escherichia coli* prevelence is greatest in the summer, with the highest incidence of *E. coli* shedding by cattle taking place in the summer months (Greenquist et al. 2005; Loneragan and Brashears 2005; Callaway et al. 2009). Higher shedder prevalence was also low, 0.21% (3 of 1,415 samples). All samples that were classified as coming from high-shedders were also positive after enrichment. Neither WDGS inclusion nor the inclusion of *L. acidophilus* combined with *P. freudenreichii* in the diet had any effect ($P > 0.10$) on *E. coli* shedding in this experiment.

Implications

Wet distiller's grains plus solubles can be an effective protein and energy source for feedlot cattle by replacing traditional ration ingredients at appropriate levels in feedlot diets. This study suggests that WDGS has a greater feeding value than DRC due to the improved performance in heifers receiving the diet containing 30% WDGS. While there

is some evidence that DFM improve cattle performance, results have been inconsistent. We observed that the inclusion of a DFM containing *L. acidophilus* combined with *P. freudenreichii* had no effect on animal performance. While some research suggests that WDGS and DFM can impact *E. coli* shedding in feedlot cattle, the prevalence of *E. coli* O157:H7 throughout the study was too low to make any inferences. Feeding 30% WDGS to feedlot heifers improved animal performance compared to the DRC based control diet.

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Table 1. Composition of experimental diets on a dry matter (DM) basis

	Wet distiller's grains plus solubles				Dry rolled corn			
Ingredient (% DM)[1]	Receiving	Step 1	Step 2	Finisher	Receiving	Step 1	Step 2	Finisher
Dry rolled corn	44.00	49.00	54.00	58.00	52.75	62.50	72.25	80.75
Wet distiller's grains	15.00	20.00	25.00	30.00	0.00	0.00	0.00	0.00
Prairie hay	17.50	12.50	10.00	6.00	17.50	12.50	10.00	6.00
Alfalfa hay	17.50	12.50	5.00	0.00	17.50	12.50	5.00	0.00
Fat	0.00	0.00	0.00	0.00	0.25	0.50	0.75	1.25
Liquid supplement	0.00	0.00	0.00	0.00	6.00	6.00	6.00	6.00
Dry supplement	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Nutrient (DM basis)								
NE_m, Mcal/kg	1.78	1.88	1.99	2.07	1.83	1.95	2.06	2.17
NE_g, Mcal/kg	1.10	1.19	1.26	1.34	1.15	1.24	1.33	1.42
Crude protein, %	14.59	15.53	16.16	17.08	14.29	13.98	13.36	12.99
Crude fat, %	4.42	4.99	5.57	6.12	4.63	5.08	5.54	6.21
NDF, %	29.66	26.44	23.77	21.13	24.75	20.03	15.85	11.69
ADF, %	18.59	15.58	12.87	10.30	16.20	12.39	8.89	5.51
Calcium, %	1.00	0.91	0.79	0.70	1.03	0.93	0.81	0.71
Phosphorus, %	0.35	0.39	0.43	0.47	0.29	0.30	0.30	0.35
Potassium, %	0.94	0.87	0.77	0.70	1.09	0.97	0.83	0.71
Sulfur, %	0.18	0.19	0.19	0.20	0.21	0.21	0.20	0.20
Rumensin, mg/kg	33.07	33.07	33.07	33.07	33.07	33.07	33.07	33.07
Tylan, mg/kg	9.92	9.92	9.92	9.92	9.92	9.92	9.92	9.92

[1]All values are presented on a dry matter basis.
[2]Nutrient composition calculated using NRC values (Nutrient Requirements of Beef Cattle 1996).

Table 2. Effects of *Lactobacillus acidophilus* Combined with *Propionibacterium freudenreichii* Fed with or without 30% Wet Distillers Grains plus Solubles on Body Weight and Average Daily Gain

	WDGS[1]		DRC[1]			P-Value		
Item	Control[2]	DFM[2]	Control[2]	DFM[2]	SEM	Diet	DFM	Diet × DFM
Body weight, kg								
Initial	303	303	303	303	20.5	0.98	0.99	0.98
d 28	338	333	333	336	18.9	0.78	0.76	0.26
d 56	381	377	376	373	18.7	0.16	0.24	0.82
d 84	426	424	419	415	20.7	0.06	0.41	0.80
d 119	479	479	475	471	21.4	0.27	0.69	0.69
Finish[3]	516	517	513	503	13.8	0.14	0.43	0.35
Carcass adjusted[4]	518	519	513	505	13.1	0.13	0.56	0.48
Average daily gain, kg								
d 1 - 28	1.07	0.90	0.86	1.04	0.06	0.57	0.89	0.01
d 29 - 56	1.61	1.64	1.61	1.39	0.09	0.15	0.26	0.15
d 57 - 84	1.59	1.67	1.53	1.48	0.09	0.09	0.90	0.40
d 85 - 119	1.55	1.62	1.65	1.66	0.07	0.38	0.59	0.67
d 120 - finish[3]	0.97	0.99	0.96	0.86	0.19	0.25	0.57	0.35
d 1 - finish[3]	1.31	1.31	1.28	1.24	0.09	0.08	0.53	0.40
Carcass adjusted[4]	1.34	1.34	1.30	1.26	0.08	0.05	0.45	0.43

[1]WDGS = Wet Distiller's Grains plus Solubles. DRC = Dry-Rolled Corn.

[2]Control treatments contained no DFM. DFM treatments contained 1×10^6 colony forming units of *Lactobacillus acidophilus* combined with 1×10^9 colony forming units of *Propionibacterium freudenreichii*.

[3]Heifers were harvested on d 132 (Heavy block), d 167 (Medium block), or d 188 (Light block).

[4]Carcass adjusted BW calculated as HCW/average dressing percent for each weight block.

Table 3. Effects of *Lactobacillus acidophilus* Combined with *Propionibacterium freudenreichii* Fed with or without 30% Wet Distillers Grains plus Solubles on Dry Matter Intake and Gain:Feed

	WDGS[1]		DRC[1]			P-Value		
Item	Control[2]	DFM[2]	Control[2]	DFM[2]	SEM	Diet	DFM	Diet × DFM
Dry matter intake, kg								
d 1 - 28	7.87	7.78	7.65	7.89	0.48	0.69	0.57	0.21
d 29 - 56	8.91	8.94	8.44	8.47	0.43	0.01	0.84	0.98
d 57 - 84	9.10	9.09	8.94	8.75	0.47	0.24	0.63	0.68
d 85 - 119	8.93	9.13	9.34	9.17	0.40	0.26	0.92	0.35
d 120 - finish[3]	8.15	8.54	8.52	8.32	0.49	0.69	0.61	0.12
d 1 - finish[3]	8.56	8.70	8.59	8.53	0.46	0.65	0.82	0.53
Gain:Feed								
d 1 - 28	0.136	0.116	0.114	0.131	0.014	0.70	0.89	0.04
d 29 - 56	0.183	0.186	0.194	0.166	0.014	0.68	0.24	0.15
d 57 - 84	0.176	0.183	0.172	0.170	0.007	0.21	0.68	0.48
d 85 - 119	0.175	0.180	0.178	0.183	0.008	0.70	0.54	1.00
d 120 - finish[3]	0.117	0.115	0.111	0.101	0.016	0.16	0.41	0.58
d 1 - finish[3]	0.150	0.149	0.147	0.143	0.003	0.19	0.39	0.65
Carcass adjusted[4]	0.155	0.154	0.152	0.147	0.003	0.10	0.32	0.63

[1]WDGS = Wet Distiller's Grains plus Solubles. DRC = Dry-Rolled Corn.

[2]Control treatments contained no DFM. DFM treatments contained 1×10^6 colony forming units of *Lactobacillus acidophilus* combined with 1×10^9 colony forming units of *Propionibacterium freudenreichii*.

[3]Heifers were harvested on d 132 (Heavy block), d 167 (Medium block), or d 188 (Light block).

[4]Carcass adjusted BW calculated as HCW/average dressing percent for each weight block.

Table 4. Effects *Lactobacillus acidophilus* Combined with *Propionibacterium freudenreichii* Fed with or without 30% Wet Distillers Grains Plus Solubles on Carcass Characteristics

	WDGS[1]		DRC[1]			P-Value		
Item	Control[2]	DFM[2]	Control[2]	DFM[2]	SEM	Diet	DFM	Diet × DFM
HCW, kg	333	333	329	324	7.08	0.13	0.56	0.47
Dressing percentage	64.3	64.5	64.2	64.2	0.00	0.53	0.81	0.87
Ribeye area, sq cm	82.2	80.7	83.5	82.0	2.69	0.28	0.23	0.98
12th-rib fat, cm	1.61	1.65	1.54	1.46	0.09	0.10	0.79	0.47
KPH, %	3.19	3.30	3.09	3.37	0.14	0.93	0.19	0.55
Marbling score[3]	404	411	418	431	14.0	0.09	0.33	0.75
Prime and Choice, %	56.6	49.3	56.6	56.3	9.78	0.64	0.61	0.64
Yield grade	2.93	3.08	2.74	2.79	0.38	0.07	0.41	0.68
Liver Score[4]	0.19	0.35	0.57	0.28	0.14	0.29	0.63	0.11

[1]WDGS = Wet Distiller's Grains plus Solubles. DRC = Dry-Rolled Corn.

[2]Control treatments contained no DFM. DFM treatments contained 1×10^6 colony forming units of *Lactobacillus acidophilus* combined with 1×10^9 colony forming units of *Propionibacterium freudenreichii*.

[3]Marbling scores: 400 = Small^{00}, 500 = Modest^{00}.

[4] Liver Score: 0 = no abscesses, 1 = A-, 2 = A, 3 = A+, 4 = telangiectasis, 5 = distoma (fluke damage), and 6 = fecal contamination.

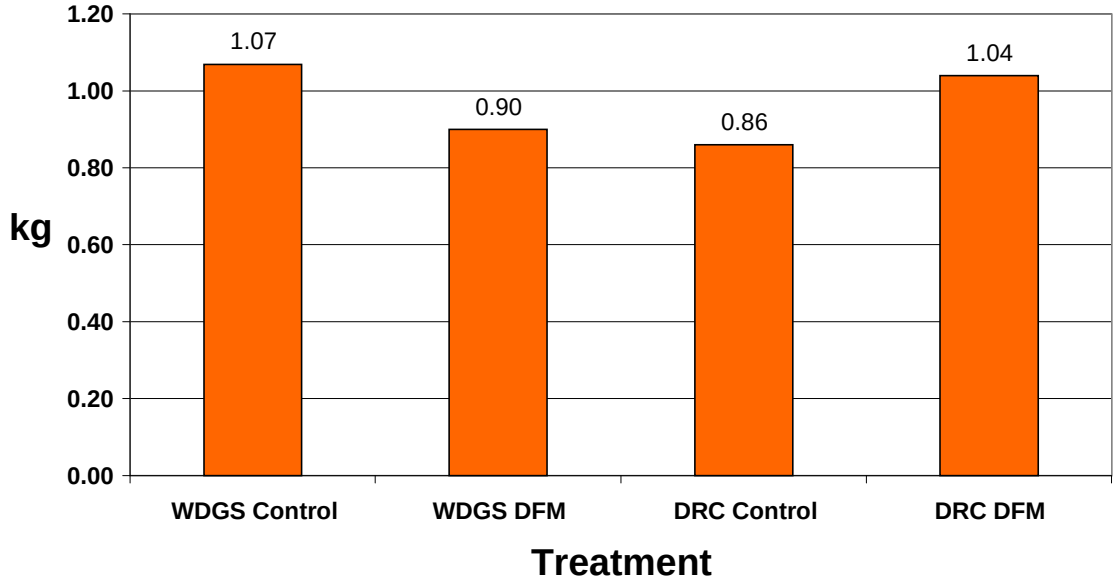

Figure 1. Graph of average daily gain for the first interval of the experiment (days 1 to 28) demonstrating the interaction of the *Lactobacillus acidophilus* combined with *Propionibacterium freudenreichii* direct-fed microbial (DFM) fed with or without 30% wet distillers grains plus solubles (WDGS) on average daily gain in kilograms from days 1 to 28. Diets consisted of the 30% WDGS based diet or the dry-rolled corn (DRC) based diet. The DFM treatment consisted of the DFM (containing 1×10^6 colony forming units of *Lactobacillus acidophilus* combined with 1×10^9 colony forming units of *Propionibacterium freudenreichii*) or the control which contained no DFM. P-values for the interval were (P = 0.57) for the diet, (P = 0.89) for the DFM, and (P = 0.01) for the diet × DFM interaction. SEM for the interval was 0.06.

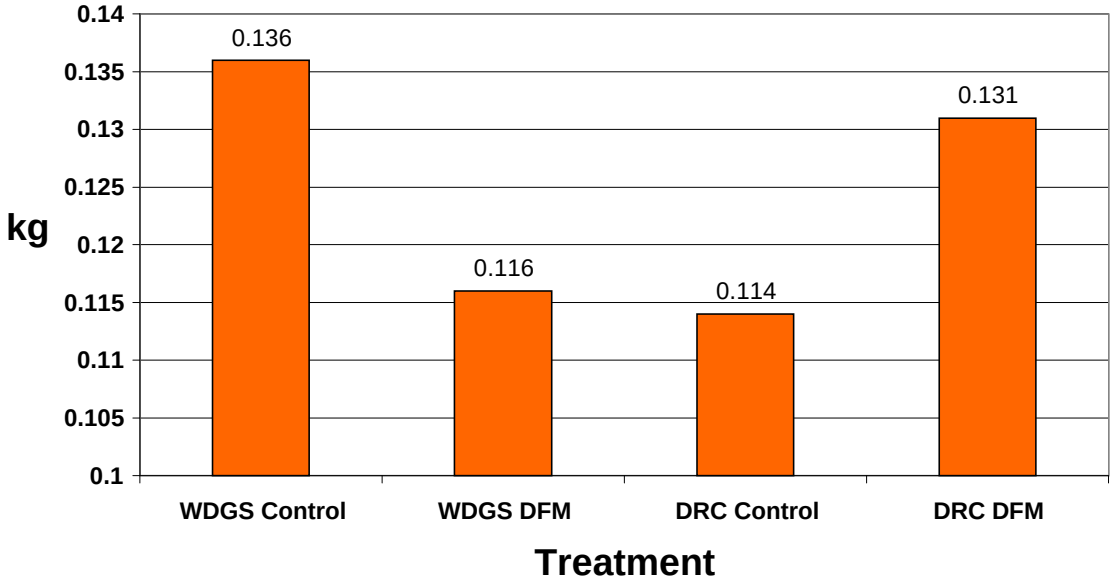

Figure 2. Graph of gain:feed for the first interval of the experiment (days 1 to 28) demonstrating the interaction of the *Lactobacillus acidophilus* combined with *Propionibacterium freudenreichii* direct-fed microbial (DFM) fed with or without 30% wet distillers grains plus solubles (WDGS) on gain:feed in kilograms. Diets consisted of the 30% WDGS based diet or the dry-rolled corn (DRC) based diet. The DFM treatment consisted of the DFM (containing 1×10^6 colony forming units of *Lactobacillus acidophilus* combined with 1×10^9 colony forming units of *Propionibacterium freudenreichii*) or the control which contained no DFM. P-values for the interval were (P = 0.70) for the diet, (P = 0.89) for the DFM, and (P = 0.04) for the diet × DFM interaction. SEM for the interval was 0.014.

VITA

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Candidate for the Degree of

Master of Science

Thesis: FEEDING A DIRECT-FED MICROBIAL TO DETERMINE PERFORMANCE, CARCASS CHARACTERISTICS, AND FECAL SHEDDING OF *ESCHERICHIA COLI* O157:H7 IN FEEDLOT HEIFERS FED WITH OR WITHOUT WET DISTILLER'S GRAINS PLUS SOLUBLES

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Abstract

Fluctuating corn prices related to increased ethanol production have had a significant impact on the cost of gain for cattle feeders that rely on corn-based diets. The inclusion of wet distiller's grains plus solubles (WDGS) in feedlot diets has become a common practice in many regions of the U.S. due to the expanded production of by-products. In addition, societal concerns over the continued use of antibiotics in production agriculture combined with an enhanced interest in disease and pathogen prevention in the food supply have led to an increased interest in use of direct-fed microbials (DFM) in growing and finishing cattle. Direct-fed microbials have been shown to improve ADG and feed efficiency, alter ruminal fermentation, and decrease fecal shedding of harmful pathogens in feedlot cattle. The objective of this experiment was to evaluate the effects of *Lactobacillus acidophilus* combined with *Propionibacterium freudenreichii* on performance, carcass characteristics, and *Escherichia coli* O157:H7 shedding in feedlot heifers fed with or without WDGS. Crossbred heifers (n = 288; initial BW = 295 ± 28 kg) were assigned to 1 of 4 treatments (12 pens per treatment; 6 heifers per pen) in a randomized complete block design with a 2 × 2 factorial arrangement of treatments. Across the feeding period, heifers fed 30% WDGS tended ($P = 0.09$) to have greater ADG and had greater carcass-adjusted ADG ($P = 0.05$) compared with heifers fed dry-rolled corn (DRC). Dry matter intake was not affected ($P = 0.65$) by diet, although carcass adjusted G:F tended ($P = 0.10$) to be improved for heifers fed WDGS. Heifers fed 30% WDGS tended ($P \leq 0.10$) to have greater fat thickness at the 12th rib, lower marbling scores, and higher yield grades. The inclusion of *L. acidophilus* combined with *P. freudenreichii* in the diet had no effect ($P > 0.10$) on performance or carcass merit in the present experiment. The incidence of *Escherichia coli* O157:H7 throughout the experiment was low, with only 18 positive samples across all sampling periods. Neither WDGS inclusion nor the inclusion of *L. acidophilus* combined with *P. freudenreichii* in the diet had any effect ($P > 0.10$) on *E. coli* O157:H7 shedding in this experiment. Feeding 30% WDGS to feedlot heifers improved animal performance compared to the DRC based control diet.

ADVISER'S APPROVAL: Dr. Clint R. Krehbiel