Management strategies to decrease antimicrobial use and liver abscesses in growing and finishing beef cattle

by

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CHAPTER I

INTRODUCTION

In recent years, feedlot production systems have seen an increase in the incidence of liver abscesses in beef cattle. Typically resulting from aggressive grain-feeding programs, liver abscesses affect cattle of all ages and types, but have the greatest economic relevance for conventional, grain-finished cattle (Nagaraja and Chengappa, 1998). In most feedlots, incidences average from 12 to 32% and are influenced by both management and dietary factors (Brink et al., 1990). Among cattle fed conventional, high-concentrate feedlot diets based on corn grain, acidotic conditions resulting in damage to the ruminal epithelium are accepted as primary predisposing factors for liver abscesses.

Typically, liver abscess severity and prevalence increase as dietary roughage levels decrease (Harvey et al., 1968; Foster and Woods, 1970; Gill et al., 1979; Bartle and Preston, 1991; Zinn and Plascienca, 1996). According to Nagaraja and Lechtenberg (2007) it is possible that the inclusion of roughage in a finishing diet promotes a more stable ruminal fermentation, decreases variation in animal feed intake, and ultimately lowers the occurrence of acidosis. Rapid increases in dietary energy caused by grain products with high rates of starch fermentation, ultimately cause fluctuations in ruminal pH and feed intake. Over time, such conditions prompt acidosis and rumenitis, allowing for the creation of microscopic pores in the rumen wall, giving bacteria the ability to escape the rumen and become sequestered in the liver (Elam, 1976; Nagaraja and Chengappa, 1998). Once in the liver, bacteria form a purulent filled sac, ultimately resulting in a liver abscess.

Liver abscesses are known to cause significant decreases in live animal performance, as well as have negative implications on overall carcass merit. The primary etiologic agents associated with bovine liver abscesses are believed to be *Fusobacterium necrophorum* and *Trueperella pyogenes* (Nagaraja and Chengappa, 1998), and more recent research suggests that *Salmonella enterica* may also play a

role (Amachawadi and Nagaraja, 2015). Within the feedlot industry, feed grade antimicrobial compounds are commonly relied upon to reduce the prevalence and severity of liver abscesses seen in feedlot cattle; the most common and most effective being tylosin phosphate. Recently, concerns regarding the use of antimicrobial compounds in food-producing animals has increased, because of the potential for unnecessary contributions to the further development of antimicrobial resistance. As the beef industry strives to meet consumer demands, alternative options are being researched as potential replacements for antimicrobial compounds. This includes the use of direct-fed microbials (DFM) as a potential replacement for feed grade additives used to reduce the incidence of liver abscesses. Liver abscesses pose a significant economic threat to the beef industry's producers, packers, and consumers, and a research need exists for antimicrobial alternatives.

CHAPTER II

REVIEW OF LITERATURE

EFFECTS OF LIVER ABSCESSES

Decreases in Live Animal Performance

Research has demonstrated that liver abscesses are known to have negative implications on live animal performance over time (Brown et al., 1975; Brink et al., 1990; Nagaraja and Lechtenberg, 2007). When abscesses are formed within the liver, the animal's metabolic efficiency is diminished, and overall performance is altered because of the resulting damage to hepatocytes. According to Nagaraja and Lechtenberg (2007), these effects can be seen in cattle with abscessed livers when evaluating growth performance data such as final body weight (BW), average daily gain (ADG), gain-to-feed ratio (G:F), and dry matter intake (DMI). Severe liver abscesses (A+) tend to decrease final live animal BW when compared to the live BW of animals with healthy livers (Brown et al, 1975; Brink et al., 1990). According to Brown et al. (1975) the presence of severe liver abscesses decreased total animal gain by 12.7% when compared to cattle with no liver abscesses. Similarly, Brink et al. (1990) reported a 16.6% decrease in total animal gain when comparing cattle with severe liver abscesses to cattle with un-abscessed livers. Cattle with liver abscesses have also been reported as having a 14% decrease in ADG and a 13% decrease in G:F (Brink et al., 1990). Overall, decreases in live animal performance as a consequence of liver abscesses, can result in detrimental decreases in animal efficiency and value.

Decrease in Carcass Performance/Merit

Liver abscesses are not only known to negatively impact cattle growth performance, but also negatively affect carcass characteristics. As a result of decreased live animal performance, severe liver abscesses can result in decreased hot carcass weights (HCW) of up to 3.6% and decreased overall carcass merit (Brink et al., 1990; Davis et al., 2007). Cattle with severe liver abscesses may also require additional

carcass trim and condemnation of the entire viscera due to the adhesion of abscesses to the animal's diaphragm and surrounding organs (Nagaraja and Chengappa, 1998). Additionally, Brink et al. (1990) observed highly significant decreases in dressing percentage (DP) for cattle with severe liver abscesses compared to cattle with healthy livers at the time of slaughter; 60.0 and 61.2%, respectively, which was attributed to trim loss. Furthermore, data has shown decreased marbling scores and decreased *Longissmus* muscle (LM) area in cattle with A+ liver abscesses compared to those with healthy livers (Brown and Lawrence, 2010). According to Brown and Lawrence (2010), gross carcass value analyses showed that carcasses with severe liver abscesses were less valuable than carcasses with normal livers. Ultimately, the presence of liver abscesses in feedlot cattle can have negative implications on overall carcass characteristics and cause significant losses in carcass value.

ETIOLOGIC AGENTS OF CONCERN

The bacterial flora of liver abscesses, both anaerobic and aerobic, has been extensively researched (Lechtenberg et al., 1988; Nagaraja et al., 1996; Tan et al., 1996). Studies conclude *Fusobacterium necrophorum* as the primary causative agent. *Fusobacterium necrophorum* is also implicated as the primary pathogen in foot rot, foot abscesses, and necrotic laryngitis (calf diptheria; Tan et al., 1996). According to Nagaraja and Chengappa (1998), *F. necrophorum* occurs in 81 to 100% of all cultured liver abscesses. While *F. necrophorum* has been isolated from liver abscesses as a single pathogen (Lechtenberg et al., 1988), it is commonly associated with other anaerobic and facultative bacteria (Scanlan and Hathcock, 1983). This includes *Trueperella pyogenes,* previously known as *Actinomyces pyogenes* (Berg and Scanlan, 1982; Lechtenberg et al., 1988), *Bacteroides* spp. (Simon and Stovell, 1971; Berg and Scanlan, 1982), *Clostridium* spp., *Pasteurella* spp. (Simon and Stovell, 1971), *Peptostreptococcus* spp. (Berg and Scanlan, 1982), *Staphylococcus* spp. (Berg and Scanlan, 1982; Lechtenberg et al., 1988), *Streptococcus* spp. (Simon and Stovell, 1971; Lechtenberg et al., 1988), and *Salmonella enterica* (Amachawadi and Nagaraja, 2015). Furthermore, research concludes that *T. pyogenes* is the second most common

pathogen isolated from liver abscesses (Berg and Scanlan, 1982; Lechtenberg et al., 1988; Nagaraja and Chengappa, 1998). Evidence also suggests that a synergistic relationship exists between *F. necrophorum* and *T. pyogenes* (Takeuchi, 1983).

Fusobacterium necrophorum

Fusobacterium necrophorum is characterized as an anaerobic, gram-negative, nonmotile, nonsporulating, rod-shaped bacterium, and is a normal inhabitant of the mammalian gastrointestinal tract (Langworth, 1977). Traditionally, the concentration of *F. necrophorum* in the rumen is in the range of 10^5 to 10^6/g of rumen content, however this concentration can be heavily influenced by the diet (Nagaraja and Chengappa, 1998). Generally, this species does not rely on carbohydrate fermentation, but rather uses lactic acid as its primary energy substrate, which is then fermented to acetate, butyrate, and proprionate (Lechtenberg et al., 1988). This would explain why increased populations of *F. necrophorum* are commonly found in cattle consuming high-grain diets, as there is an increased availability of lactate in the rumen (Nagaraja and Chengappa, 1998).

According to Langworth (1977), *F. necrophorum* is classified into four biotypes: A, B, AB, and C. Biotypes A and B are most commonly isolated from bovine liver abscesses and differ in their cellular morphology, growth patterns, colonization characteristics, extracellular enzymes, hemagglutination properties, hemolytic activities, leukotoxin activities, chemical compositions of lipopolysaccharides (**LPS**), and virulence (Langworth, 1977; Scanlan and Hathcock, 1983; Tan et al., 1996; Nagarja et al., 1996). Biotype AB is rarely isolated from bovine liver abscesses and has intermediate characteristics of both biotypes A and B (Berg and Scanlan, 1982; Scanlan et al., 1982). While biotype C has been identified as structurally similar to biotypes A and B, it is non-pathogenic and does not produce similar virulence factors, therefore, it has been reclassified as *Fusobacterium pseudonecrophorum* (Shinjo et al., 1990). Among *F. necrophorum* biotypes A and B, according to Tan et al. (1996), the production of toxins and other virulence factors

among the two biotypes promotes an environment suitable for the development of liver abscesses.

In the pathogenesis of *F. necrophorum,* research has identified several associated virulence factors relevant to the bacterium's survival and proliferation within the host (Tan et al., 1996; Nagaraja and Chengappa, 1998). These factors include: leukotoxin, endotoxic LPS, hemolysin, hemagglutinin, capsule, adhesions or pili, platelet aggregation factor, dermonecrotic toxin, and several extracellular enzymes, including proteases and deoxyribonucleases (Tan et al., 1996; Nagaraja et al., 2005). Of the virulence factors that are implicated in the pathogenesis of *F. necrophorum,* research suggests that leukotoxin production is the major virulence factor involved in fusobacterial infection among animals (Narayanan et al., 2002). According to Tan et al. (1994), *F. necrophorum* leukotoxin is a secreted protein that is cytotoxic to neutrophils, macrophages, hepatocytes, and feasibly, to ruminal epithelial cells. Evidence suggests that *F. necrophorum* biotype A produces more leukotoxin that biotype B (Berg and Scanlan, 1982; Scanlan et al., 1982; Tan et al., 1992). This evidence would support the findings of Lechtenberg et al. (1988) in which *F. necrophorum* biotype A was isolated from 71 to 95% of liver abscesses, while biotype B was isolated from only 5 to 29% of liver abscesses. A correlation between toxin production and the ability to induce liver abscesses in laboratory animals indicates the importance of leukotoxin on the severity of *F. necrophorum* infections (Emery et al., 1986).

Truperella pyogenes

Truperella pyogenes is the second most common pathogen isolated from bovine liver abscesses (Berg and Scanlan, 1982; Lechtenberg et al., 1988; Tan et al., 1996; Nagaraja and Chengappa, 1998) and is characterized as a gram-positive, rod-shaped, and facultatively-anaerobic bacterium (Amachawadi and Nagaraja, 2016). *Truperella pyogenes* found in liver abscesses is commonly cultured from the ruminal wall rather than from ruminal contents (Narayanan et al., 1998). Because of the availability of oxygen directly from the blood circulation within the ruminal wall and

T. pyogenes niche as a facultative anaerobe, the ruminal wall provides an optimal environment for growth and proliferation (Amachawadi and Nagaraja, 2016). According to Tadapalli et al. (2009), the frequent association of *T. pyogenes* and *F. necrophorum* is a result of a nutritional and pathogenic synergy that exists between species. *Truperella pyogenes* creates an anerobic condition through its use of oxygen, providing an optimal environment for the survival and proliferation of *F. necrophorum.* Additionally, lactic acid is a waste product of *T. pyogenes*, in turn, supplying the primary energy substrate for *F. necrophorum*.

According to Billington et al. (1997), the primary virulence factor associated with *T. pygoenes* is a hemolysin, called pyolysin, that is cytoxic to polymorphonuclear cells (white blood cells). Additionally, other virulence factors involved in the adherence, colonization, and pathogenicity of *T. pyogenes* include proteases, deoxyribonucleases, neuraminidases, and extracellular matrix binding protein (Jost and Billington, 2005). While *T. pyogenes* has been isolated as a single organism from liver abscesses (Nagaraja and Lechtenberg, 2007), it is most commonly isolated in conjunction with *F. necrophorum.* Research has indicated that the leukotoxin produced by *F. necrophorum* may allow *T. pyogenes* to survive in the liver and ultimately aid in abscess formation (Lechtenberg et al., 1993).

Salmonella enterica

In addition to *F. necrophorum* and *T. pyogenes,* recent research has isolated *Salmonella enterica* from bovine liver abscesses, suggesting it may have an influence on the onset and severity of liver abscesses (Amachawadi and Nagaraja, 2015). *Salmonella enterica* is classified as a gram-negative, motile, flagellate, facultative intracellular pathogen that possesses the ability to adapt to diverse environments, including fluctuations in oxygen concentrations (Amachawadi and Nagaraja, 2015). According to Yamamoto and Droffner (1985), *Salmonella* bacteria are capable of robust growth under anaerobic conditions, making the rumen an ideal environment for growth. At this time, the role of *S. enterica* as an etiologic agent is unknown. As a result of its motility and flagellate qualities that allow it to move freely, it is possible

that *S. enterica* may have entered after *F. necrophorum* initiated the abscess in the liver. According to Amachawadi and Nagarja (2015), it is possible that *S. enterica* present in the gastrointestinal tract could cross the epithelial barrier into portal circulation, and become trapped in the portal capillary system of the liver to initiate infection. Such entry through the gut epithelium could be warranted through inflammation or ruminal acidosis (Nagaraja and Lechtenberg, 2007). Additionally, *S. enterica* has been isolated from the lymph nodes of cattle at the time of slaughter (Arthur et al., 2008), suggesting the lymphatic system as a potential source of pathogenesis. Further research is needed to determine the exact role of *S. enterica* in the bovine liver abscess complex.

ECONOMIC INFLUENCE OF LIVER ABSCESSES ON THE FEEDLOT INDUSTRY

In the United States, liver condemnation caused by abscesses is among one of the greatest economic liabilities to cattle producers and packers (Nagaraja and Lechtenberg, 2007). While liver losses account for significant financial implications, the greatest financial loss comes from reductions in both animal growth performance and overall carcass merit, as discussed above. According to Brown and Lawrence (2010), the most severe liver abscesses can reduce the value of beef carcasses by as much as $88 per animal. Furthermore, contamination of beef carcasses during slaughter because of ruptured abscesses, can accrue additional costs relative to lost time and labor (Nagaraja and Chengappa, 1998). In summary, liver abscesses pose a major economic threat to the beef industry, therefore, the need to effectively reduce and manage liver abscesses in beef cattle is significant to the overall financial wellbeing of the feedlot industry.

TYLOSIN PHOSPHATE

Overview

In the feedlot industry, one of the most common methods used to minimize the incidence of liver abscesses involves the continuous feeding of antimicrobial compounds; the most common and most effective being tylosin phosphate. Since the

feeding of tylosin phosphate, a macrolide antibiotic, is routine in the United States feedlot industry, extensive research exists regarding the resistance and susceptibility of *Fusobacterium necrophorum* (Nagaraja and Chengappa, 1998). According to Lechtenberg et al. (1998), *F. necrophorum* is susceptible to penicillins, tetracyclines, lincosamides, and macrolides, while being resistant to aminoglycosides, peptides, and ionophores.

Mechanism of Action

Tylosin phosphate, classified as a macrolide antimicrobial compound, is primarily effective against gram-positive bacterial species, however, has proved to be effective against gram-negative, *F. necrophorum* (Berg and Scanlan, 1982). According to Nagaraja and Chengappa (1998), tylosin is believed to act by inhibiting the proliferation of *F. necrophorum.* Nascent peptide chains become blocked from entering the large ribosomal subunit, causing the dissociation of peptidyl-tRNAs from the ribosome, ceasing the synthesis and proliferation of functional proteins within the bacterium (Tenson et al., 2003); whereby, causing death of the bacterium because of lack of functional protein. Earlier research conducted by Gingerich et al. (1977) found that tylosin phosphate could be partially absorbed from the gut and detected in blood serum samples. While it is possible that tylosin may also act upon microbial populations in the liver, it is accepted that it predominantly has effects in the rumen (Gingerich et al., 1977; Nagaraja and Chengappa, 1998). According to Nagaraja et al. (1999), when feeding high-concentrate diets, the dietary inclusion of tylosin phosphate has been reported to limit the increase of *F. necrophorum* populations.

Decreased Prevalence of Liver Abscesses

Although alternative methods exist to prevent and control the incidence of liver abscesses in the feedlot industry, tylosin phosphate has been noted as the most effective at reducing the prevalence of liver abscesses in beef cattle. Therefore, it has become a common component of many finishing cattle diets. Subsequently, as a result of decreased liver abscesses, producers have also seen significant improvements in

animal performance with the inclusion of tylosin phosphate in finishing cattle diets. Several studies have concluded that by including tylosin in the diet, there is a reduction in abscess incidence by 40 to 70% (Brink et al., 1990; Bartle and Preston, 1991; Tan et al., 1994). According to Vogel and Laudert (1994) in a summary of 40 trials involving cattle from all major cattle feeding areas, feeding tylosin at the recommended 90 mg/animal/d decreased the incidence of liver abscesses by as much as 73%. Additionally, research has reported that including monensin alone in a finishing diet did not reduce the incidence of liver abscesses, however the inclusion of tylosin, in addition to monensin, decreased the overall presence of abscesses at the time of slaughter (Meyer et al., 2013). According to Meyer et al. (2013), the addition of tylosin to a diet containing wet distillers grains plus solubles (WDGS) and monensin decreased total liver abscesses by 80.4% (Exp. 1) and 61.0% (Exp. 2) when compared to diets containing only WDGS. The benefits of feeding tylosin phosphate make it a viable method to control the incidence of liver abscesses in finishing beef cattle, while also helping to mitigate the economic liability they place upon the beef industry.

Economic Impacts of Tylosin Phosphate

Feeding tylosin phosphate has been reported as beneficial for maintaining animal performance and reducing the incidence of liver abscesses, mitigating the financial threat posed to both producers and packers. The feeding value of tylosin phosphate has been estimated at $0.01/animal/d and increases final live BW by approximately 4 to 5 kg (Vogel, 2019). Assuming fed steer BW at 621 kg at the time of slaughter, average dressing percentage of 63.48%, and live steer price of $2.66/kg (USDA, 2021), by feeding tylosin phosphate in the diet, there is an additional price increase of $6.77/animal, versus animals not fed tylosin phosphate. In 2013, it was estimated that 71.2% of all finishing cattle had received tylosin phosphate during feedlot placement through the finishing phase (USDA, 2013). Additionally, according to the USDA (2021), there were an estimated 638,000 beef animals slaughtered the week of May 8, 2021. Furthermore, assuming 454,256 animals slaughtered that week

received tylosin phosphate and with an increased profit of $6.77/animal, by feeding tylosin phosphate, fed beef profits equate to $3,075,313 weekly or $159,916,282 annually.

ANTIMICROBIAL RESISTANCE

Overview

In United States feedlot production systems, the continuous feeding of antimicrobial compounds, such as tylosin phosphate, is a common practice to minimize the incidence of liver abscesses. However, in recent years, the beef industry has come under great scrutiny by consumers, animal advocates, and regulatory agencies to reduce the use of antimicrobial compounds within the beef industry. In the 21st century, the potential for antimicrobial resistance (AMR) is one of the largest global human-health threats (CDC, 2019). Annually in the United States, the CDC (2019) reports approximately 2.8 million infections and 35,000 deaths related to antimicrobial resistance. While tylosin phosphate is not approved for use in human medicine, it is categorized by the Food and Drug Administration (FDA) as a medically important antimicrobial (Schmidt et al., 2020). The inclusion of tylosin in finishing cattle diets is not directly linked to a specific antimicrobial-resistant pathogen threatening human health, therefore, any concerns regarding tylosin phosphate use during beef cattle production are generalized (Hoelzer et al., 2017). However, there is still a valuable research need for alternative options to control the prevalence of liver abscesses in the future, as reducing antimicrobial use in food-producing animals is desirable.

Significance of Antimicrobial Resistance

Antimicrobial resistance is an inevitable consequence of the use of antimicrobials, as microorganisms are continuously evolving under selective pressure (Hand, 2013). However, according to Hand (2013), the rate and extent at which resistant organisms propagate, is determined primarily by antibiotic consumption in humans and animals. To preserve the effectiveness of currently available

antimicrobials, the World Health Organization (WHO) developed criteria to rank antimicrobials based on their importance in human medicine (Powers, 2004; Collignon et al., 2009). To be deemed critically important, antimicrobials are evaluated based on their ability to treat serious infections with limited treatments, an infection's ability to be transmitted from non-human sources, or the bacteria's ability to acquire resistant genes from non-human sources (Scott et al., 2019). Antimicrobials classified as critically important to human medicine include aminoglycosides, ansamycins, carbapenems, cephalosporins, glycopeptides, glycylcyclines, lipopeptides, macrolides, monobactams, oxazolidinones, penicillins, phosphonic acid derivatives, polymyxins, and quinolones. The increasing threat of AMR compromises advances in modern medicine and livestock production, and therefore, strategies to minimize resistance are crucial for improving the longevity of human and animal health.

Feed Grade Antimicrobial Use in the Beef Industry

Within beef cattle production, antimicrobials are commonly used for the therapeutic treatment of infections caused by bacteria and other microorganisms that are not combatable with vaccines, bacterins, or alternative treatment therapies (Cameron and McAllister, 2016). Furthermore, compromised animal health creates a significant economic pressure for antimicrobial use to prevent and treat infectious disease (Cameron and McAllister, 2016). Under the introduction of the Veterinary Feed Directive (VFD) in the United States in 2015, the in-feed inclusion of medically important antibiotics, without written veterinary direction, for the purpose of improving food animal production was eliminated (FDA, 2015). In dairy and beef production systems, the use of medically important medicated feed additives such as tetracyclines and macrolides are commonplace (Quinn, 2020). Samuelson et al. (2016), in a survey of feedlot consulting nutritionists, reported that 83.4% of producers used a medicated feed additive to control liver abscesses and 43.2% used a medicated feed additive for managing foot health. In a survey of 22 feedlots, Hope et al. (2020) reported that in 2016 and 2017, the in-feed inclusion of a macrolide- or tetracycline-based feed additive was 42.4 and 28.0 mg/kg for each kg of live animal body weight,

respectively. The continued judicious use of feed grade antimicrobials in beef cattle production is crucial to maintaining the best interest of both business operations and animal well-being.

Causes of Antimicrobial Resistance

Antimicrobial resistance is a multi-faceted and complex problem influenced by a variety of factors. The World Health Organization (WHO; 2015) contributes increases in AMR to: the over-prescription of antibiotics, over-use of antimicrobials in livestock and aquaculture, poor infection control in medical settings, lack of hygiene, and poor sanitation. According to Collignon (2015), the largest causes of AMR include the total volume of antimicrobials used, and the spread of resistant microorganisms and genes encoding for resistance. Failure to effectively diagnose infection, identify causative agents, and confirm antimicrobial susceptibility of a given pathogen, increases the over-application of antibiotics in clinical practice (Michael et al., 2014). In extreme cases requiring immediate medical intervention, a combination of antimicrobials may be administered simultaneously because of the onset of life-threatening symptoms; thus, increasing selective pressure for AMR in pathogenic and non-pathogenic bacteria (Michael et al., 2014). As the global population continues to increase and remains largely connected, the spread of resistant microorganisms and transfer of genetic material encoding for AMR to humans, animals, and the environment remains relevant (Michael et al., 2014; Collignon, 2015; Collignon and Beggs, 2019). While the connection between AMR in beef cattle production systems and human medicine remains investigated, antimicrobial use in food animal production has been identified as a contributor to the development of antimicrobial resistance (Hoelzer et al., 2017).

Primary Resistant Bacteria of Concern

Antimicrobial resistance within bacteria, viruses, and parasites causes approximately 700,000 deaths annually and is predicted to cause approximately 10 million deaths annually by 2050 (O'Neill, 2016). Because of their ability to carry

multidrug resistance, the primary resistant bacteria of concern include *S. enterica* and *Escherichia coli*. In brief, as part of the Enterobacteriaceae family, *S. enterica* are gram-negative, facultative anaerobes with a unique ability to adapt to a multitude of environments (Sanderson, 1976; Amachawadi and Nagaraja, 2015). Additionally classified as a member of the Enterobacteriaceae family, *E. coli* are commensal, rod-shaped, gram-negative, facultative anaerobes (Sanderson, 1976). Multidrug resistance is defined as the simultaneous resistance of a microorganism to structurally unrelated antimicrobial drugs (Pastan and Gottesman, 1991). While the development of multidrug resistance naturally occurs as microorganisms adapt to their ever-changing environment, the inappropriate use of antimicrobials encourages the further development of multidrug resistance over time (Tanwar et al., 2014).

As the prevalence of AMR *Salmonella* and *E. coli* continues to increase, the effectiveness of available antimicrobials to treat infections decreases (Poirel et al., 2018; CDC, 2019). According to Alcaine et al. (2007), *Salmonella* possesses multidrug resistance to aminoglycosides, beta-lactams, phenicols, quinolones, fluoroquinolones, tetracyclines, sulfonamides, and trimethoprim. *Escherichia coli* is intrinsically susceptible to most clinically relevant antimicrobials, however, possesses multidrug resistance to aminoglycosides, beta-lactams, phenicols, quinolones, fluoroquinolones, fosfomycin, tetracyclines, sulfonamides, trimethoprim, and polymyxins (Poirel et al., 2018). Implicated as a predominate reservoir of genes encoding for AMR, *E. coli* readily exchanges genetic material with other bacterial species (Hartl and Dykhuizen, 1984; Blake et al., 2003). The transfer of genetic material between *Salmonella* and *E. coli* by horizontal gene transfer serves as a potential reservoir for the further spread of AMR (Fricke et al., 2009; Stecher et al., 2012; Colavecchio et al., 2017).

DIRECT-FED MICROBIALS

Overview

Within the food-producing livestock industry, concerns regarding the use of antimicrobial compounds have increased in recent years. Therefore, the interest in

DFM as a potential antimicrobial replacement has increased. Direct-fed microbials are classified as microbial based feed additives that inhibit gastrointestinal infection and provide optimal microbial environments within the digestive tract (Seo et al., 2010). According to Krehbiel et al. (2003) bacteria used as DFM are defined as single or mixed cultures of live organisms, which, when fed to animals, offer benefits to the host. It is believed that certain bacterial-based DFM could have beneficial effects in the rumen, such as decreasing the potential for ruminal acidosis (Krehbiel et al., 2003). While used as a valuable means for inhibiting infection of the gastrointestinal tract, DFM have also been noted as beneficial at improving feed efficiency and increasing daily gain in feedlot cattle. Research conducted by Huck et al. (2000) concluded that feeding bacterial DFM to feedlot cattle resulted in a 2.5 to 5% increase in daily gain and a 2% improvement in feed efficiency, while generally increasing carcass weight by 6 to 7 kg.

Mechanism of Action

Direct-fed microbials are believed to compete with and inhibit the growth of pathogens, stimulate immune system function, and maintain balance of microbial environments within the gastrointestinal tract. However, basic mechanisms of action are not well defined or clearly understood. While the mode of action is not fully understood, according to Krehbiel et al. (2003), DFM as a tool to improve overall animal health relies on adhesion, colonization, inhibitory action, and stimulation of immune function, post-ruminally. Additionally, bacterial DFM functioning primarily in the rumen, are dependent upon species or combination of species, to adequately determine mechanistic models. Ruminally active bacterial DFM can increase ruminal propionate concentrations and decrease the area below subacute ruminal pH, suggesting more efficient energy utilization and a reduction in ruminal acidosis (Krehbiel et al., 2003). Further research is needed to understand and describe the mode of action of DFM fed to ruminant animals.

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CHAPTER III

USING A NOVEL DIRECT-FED MICROBIAL AS AN ALTERNATIVE FOR TYLOSIN PHOSPHATE TO CONTROL LIVER ABSCESSES AND DECREASE ANTIMICROBIAL USE IN FINISHING BEEF STEERS

ABSTRACT

Objective:

Our objective was to evaluate the use of a novel direct-fed microbial as a viable alternative to antimicrobials to decrease liver abscesses in feedlot cattle.

Materials and Methods:

Angus beef steers (n = 240; initial BW = 263 ± 18.0 kg) were used in a randomized complete block design comprised of 3 BW blocks and 3 pen replications per treatment. Experimental treatments were randomly assigned to pen within BW block and consisted of: 1) negative control, dietary supplement contained no tylosin phosphate (Tylan-100; Elanco Animal Health, Greenfield, IN; NCON); 2) positive control, dietary supplement contained tylosin phosphate (PCON); 3) positive control with tylosin phosphate removed the last 65 d of the feeding period (PCONW); 4) novel direct-fed microbial fed at 1 g mixture/animal with 1×10^{11} CFU/g (DFM).

Results and Discussion:

By design, initial BW did not differ ($P = 0.79$) among treatments, and at the end of the 59-d receiving period, there were no differences in final live BW ($P = 0.25$). From d 0 to d 30, ADG, DMI, DMI as a percentage of BW, and G:F did not differ ($P \geq 0.21$). Likewise, the ADG, DMI, DMI as a percentage of BW, and G:F, from d 31 to d 59, did not differ ($P \geq 0.30$). In the overall receiving period from d 0 to d 59, there were no differences in ADG, DMI, DMI as a percentage of BW, or G:F ($P \geq 0.20$). During the finishing period, live- and carcass-adjusted final BW did not differ ($P \geq 0.57$) among treatments. For the overall finishing period, there were no differences in ADG, DMI, DMI as a percentage of BW, or G:F ($P \geq 0.17$). Carcass-

adjusted ADG, DMI as a percentage of BW, and G:F also did not differ ($P \geq 0.16$). Across treatments, no differences in HCW were noted ($P = 0.84$). Dressing percentage, marbling score, longissimus dorsi (LM) area, 12th-rib fat thickness, and calculated yield grade (YG) were not different among dietary treatments ($P \geq 0.32$). Liver abscess incidence and severity were not affected by dietary treatments ($P \geq 0.13$).

Implications and Applications:

The inclusion of a novel direct-fed microbial in finishing cattle diets did not affect growth performance, carcass characteristics, or the development of liver abscesses. Given the limited existing literature in beef cattle, further research evaluating the supplementation of *B. licheniformis* as a direct-fed microbial to decrease liver abscesses is warranted.

INTRODUCTION

Liver abscesses present at harvest continue to increase in the fed beef industry. A recent liver abscess survey indicated that incidence averages 20.3% overall (fed beef steers = 18.2%; fed beef heifers = 19.1%; fed Holsteins = 25.0%; Herrick, 2018) and can be influenced by multiple factors. Fluctuations in feed intake and subsequent ruminal pH, are commonly the result of rapid increases in dietary energy, caused by grain products with fast rates of starch fermentation. Over time, such conditions prompt acidosis and rumenitis, creating microscopic pores in the rumen wall, allowing bacteria to escape the rumen, and become sequestered in the liver (Elam, 1976; Nagaraja and Chengappa, 1998). Once in the liver, bacteria form a purulent discharge filled sac, ultimately resulting in a liver abscess. The primary etiologic agents of concern are *Fusobacterium necrophorum*, *Truperella pyogenes* (Nagaraja and Chengappa, 1998), and more recently, *Salmonella enterica* (Amachawadi and Nagaraja, 2015).

At feedlot arrival, newly received beef cattle often experience a variety of stressors, such as weaning, transport, comingling in combination with pathogen exposure, vaccination, dehydration, nutrient deficits, dehorning, and castration.

Exposure to such stressors can have negative implications on the rumen microbiome and microbial population of the lower gastrointestinal tract, resulting in increased morbidity, decreases in growth performance, and subsequent increases in death loss. According to Krehbiel et al. (2003), the administration of a bacterial direct-fed microbial (DFM) to feedlot cattle may mitigate these changes in the microbial population.

Feed grade antimicrobial compounds are used to decrease the prevalence of liver abscesses; the most common being tylosin phosphate. Classified as a macrolide antimicrobial, tylosin phosphate acts by inhibiting the proliferation of ruminal *F. necrophorum* (Nagaraja and Chengappa, 1998). Alternatives to antimicrobials are appealing as they decrease antimicrobial use in meat animals. Direct-fed microbials can provide optimal microbial environments within the digestive tract (Seo et al., 2010), and have the potential to replace feed grade antimicrobials to control liver abscesses in the future. Therefore, a novel DFM containing a mixed culture of live rumen bacteria, may inhibit the growth of ruminal *F. necrophorum*, decreasing the prevalence of liver abscesses at harvest. The objective of this study was to evaluate the use of a novel DFM as a viable alternative to antimicrobials to decrease liver abscesses in feedlot cattle.

MATERIALS AND METHODS

All experimental procedures for animal care and management were approved by the Texas Tech Animal Care and Use committee (ACUC #20040-04). The experiment was conducted from November 2021 to July 2022 at the Texas Tech University Burnett Center for Beef Cattle Research and Instruction 6 miles East of New Deal, TX.

Animal Arrival and Processing

Two-hundred forty Angus beef steers (n = 240; initial BW = 263 ± 18.0 kg), were sourced from multiple auction markets in Southern Missouri and transported approximately 1,047 km, and comingled at an order buyer facility near Stratford, TX. Three days later, cattle were transported approximately 309 km to the Burnett Center

on November 12, 2021 (d -1). At arrival, animals were individually weighed to an accuracy of ± 0.91 kg in a hydraulic squeeze chute calibrated with 454 kg of certified weigh cells before weighing (Silencer hydraulic squeeze chute; Moly Manufacturing, Lorraine, KS). Steers were given an individually identifiable ear tag and administered metaphylaxis with tildipirosin at 22 mg/kg of BW (Zuprevo; Merck Animal Health, Kenilworth, NJ). Furthermore, steers were vaccinated against infectious bovine rhinotracheitis virus, bovine viral diarrhea virus (types 1 and 2), bovine parainfluenza-3 virus, bovine respiratory syncytial virus (Vista 5 SQ; Merck Animal Health, Kenilworth, NJ), clostridial pathogens (Vision 7 with Spur; Merck Animal Health, Kenilworth, NJ), and *Mycoplasma bovis* (Myco-B ONE DOSE; American Animal Health Inc., Grand Prairie, TX). Additionally, steers were administered fenbendazole (Safeguard; Merck Animal Health, Kenilworth, NJ) and ivermectin (Vetrimec pour-on; Vet One, Boise, ID) for the treatment of internal and external parasites. Each steer received an implant containing 12 mg zeranol (Ralgro; Merck Animal Health, Kenilworth, NJ) before being placed in a soil-surfaced pen, where they were provided ad libitum access to water, long-stem grass hay, and a 74% concentrate receiving diet (Table 1) fed at 1% of BW. The day after processing (on d 0), steers were sorted by d -1 BW into experimental treatment groups with treatments randomly assigned to pen. Body weight was collected on d 0 and averaged with d -1 BW to calculate initial BW.

During the receiving period, all cattle were housed in soil-surfaced outdoor pens with partial shade. On d 30, cattle were revaccinated against infectious bovine rhinotracheitis virus, bovine viral diarrhea virus (types 1 and 2), bovine parainfluenza-3 virus, bovine respiratory syncytial virus (Vista 5 SQ; Merck Animal Health, Kenilworth, NJ) and clostridial pathogens (Vision 7 with Spur; Merck Animal Health, Kenilworth, NJ). On d 59, all cattle were reimplanted with 200 mg of trenbolone acetate and 40 mg estradiol (Revalor-XS; Merck Animal Health, Kenilworth, NJ).

Experimental Treatments

Throughout the receiving period, 4 dietary treatments were arranged as a randomized complete block design with 3 BW blocks, where treatments were replicated one time within each block (n = 12 pens total). Experimental treatments

were randomly assigned to pen within BW block and consisted of: 1) negative control, dietary supplement contained no tylosin phosphate (Tylan-100; Elanco Animal Health, Greenfield, IN; NCON); 2) positive control, dietary supplement contained tylosin phosphate (PCON); 3) positive control with tylosin phosphate removed the last 65 d of the feeding period (PCONW); 4) novel direct-fed microbial fed at 1 g mixture/animal per day at 1×10^{11} CFU/g (DFM).

Steers were transitioned from the 74% concentrate receiving diet to the final diet using a 4-step process of increasing concentrate (74, 76, 81, and 88% concentrate diets; Table 1). Each interim diet was fed for 7 d. By December 20, 2021 (d 37), all steers had been fully transitioned to the final diet (Table 2). After transitioning to the final diet, the bulk density of steam-flaked corn (**SFC**) was transitioned from 360 g/L (28 lb/bu) to 309 g/L (24 lb/bu) using a 3-step process (360, 335, and 309 g/L) over 21 days. Each interim SFC bulk-density was fed for 7 d.

After the completion of the receiving period on d 59, all cattle were sorted to partially slatted concrete pens to begin the finishing period. Previous dietary treatments were maintained, for example, cattle in the PCON treatment remained in the PCON treatment.

Housing and Animal Management

For the duration of the study, all steers were provided with *ad libitum* access to fresh water. The feed bunks were assessed daily at 0730 h, and feed was delivered daily at 0800 h. Bunks were managed to achieve *ad libitum* intake and to allow less than 0.45 kg of orts at the time of feeding. All diets were mixed in a paddle-type mixer (1.3 m^3 Marion paddle mixer) and delivered via a tractor-pulled feed wagon (Rotomix 84-8 wagon mixer; Rotomix, Dodge City, KS; scale accuracy $\pm$ 0.45 kg). Diets were formulated to meet nutrient requirements (NASEM, 2016) for growing and finishing beef cattle. All dietary supplements included 24 g/ton monensin sodium (Rumensin-90; Elanco Animal Health, Greenfield, IN). For experimental treatments PCON and PCONW, dietary supplements additionally included 8 g/ton tylosin phosphate (Tylan-100; Elanco Animal Health, Greenfield, IN). For the experimental DFM treatment, the DFM mixture was included in the dietary supplement with a ground corn carrier. For

consistency, experimental treatments NCON, PCON, and PCONW contained the ground corn carrier without the inclusion of the DFM mixture.

During the finishing period, on February 21, 2022 (d 100), yellow grease was removed from the finishing diet and replaced with Sweet Bran to maintain a least cost diet. All dietary treatments were sampled 3-times each week throughout the study and composited by week. Each weekly composite sample was divided and subsampled for further laboratory analyses. The first composite subsample was used to determine dry matter (DM) in a forced-air oven at 100˚C for 24 h. Weekly DM was used to calculate dry matter intake (DMI). The second subsample of the weekly composite was used for the analysis of crude protein, starch, ADF, NDF, ether extract, Ca, and P concentration were determined from chemical analysis (ServiTech Laboratories, Amarillo, TX). On d 128, steers were readministered ivermectin (Vetrimec pour-on; Vet One, Boise, ID) for the treatment of internal and external parasites.

Isolation and Growth of the DFM

The DFM strain used in this study was developed in collaboration with the University of Nebraska-Lincoln. The isolate was identified from the rumen as part of an isolate library using different culture media and isolation strategies. The resulting isolate was identified through functional screening of the isolate library using *Streptococcus bovis* and *Fusbacterium necrophorum necrophorum* as described by Bartenslager (2020). The identified isolate was commercially grown by Envirozyme Biotech (Bowling Green, OH) and was fed at 1×10^{11} CFU/animal per day.

Carcass Data

Steers were fed to a similar degree of finish by visual appraisal before transport to a commercial abattoir for harvest. Cattle were harvested on 2 dates (blocks 2 and 3 were harvested after 162 d on feed and block 1 was harvested after 183 d on feed). Individual carcass measurements were collected by plant personnel using the image analysis system. Liver scores were collected by Texas Tech University personnel. Liver scores were as follows: normal = no abscess, edible; A− = 1 to 2 small abscesses or inactive scars; A = 1 to 2 large abscesses, or multiple small abscesses; or

A+ = multiple large abscesses (Brink et al., 1990; Brown and Lawrence, 2010). Carcass quality and yield grades were determined by plant personnel using the E+V image analysis system (VBG2000, e+v technology, Oranienburg, Germany). Individual carcass measurements of ribeye area, 12th rib fat thickness, and marbling score were obtained from the E+V camera grading system (VBG2000, e+v technology, Oranienburg, Germany).

Calculations

Both average daily gain (ADG) and gain to feed ratio (G:F) were calculated on a live BW and carcass-adjusted basis. Carcass-adjusted final BW was calculated by dividing hot carcass weight (HCW) by the overall dressing percentage (DP; 64.98%). Average daily gain and carcass-adjusted ADG was calculated by subtracting initial BW from final BW, then divided by days on feed. The G:F was calculated as the quotient of ADG divided by daily DMI. Gain-to-feed was carcass-adjusted by dividing carcass-adjusted ADG by overall DMI . Empty body fat and adjusted final shrunk BW at 28% empty body fat was estimated from the equations of Guiroy et al. (2001).

Statistical Analysis

Six steers were removed from the study (4 mortalities unrelated to treatment and 2 for lameness) and excluded from the receiving period data (4 steers from NCON and 2 steers from PCON). Five additional steers were removed from the study (lameness or injury) after d 59 and excluded from the finishing period data (2 steers from NCON, 1 steer from PCON, and 2 steers from DFM). One pen of cattle (n = 4), from experimental treatment DFM, was removed from the study (chronically depressed DMI) and excluded from the finishing period data. The Kenward Roger adjustment was used to correct the degrees of freedom for unequal experimental units among treatments.

For all dependent variables, pen was the experimental unit. All growth performance and continuous carcass variables were analyzed using PROC MIXED in SAS (SAS Institute Inc., Cary, NC). The fixed effect of dietary treatment and the random effect of BW block were included in the model. Liver score and categorial

carcass data were analyzed as a binomial proportion using PROC GLIMMIX in SAS, where dietary treatment was the fixed effect and BW block was the random effect. For all analyses, significance was determined at $P \leq 0.05$, with tendencies being defined between $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

The use of bacterial DFM for humans and animals has been studied since the mid-1950s (Krehbiel et al., 2003). Within the cattle industry, DFM have been evaluated for more than 30 yr for promoting optimal animal health, increasing growth performance, and increasing gain:feed (LeJeune and Wetzel, 2007). McAllister et al. (2011) reported most studies using DFM in cattle production systems have focused on the use of one or more lactic acid-producing bacteria. The novel DFM used in the present study contains isolates of *Bacillus* spp., with the subspecies being *Bacillus licheniformis* isolated from the rumen using specific media as described previously by Bartenslager (2020). *Bacillus licheniformis* is a gram-positive, spore-forming, facultative anaerobe known for its ability to produce protease enzymes (Doto and Liu, 2011). In the present study, *B. licheniformis* was selected because of its ability to decrease *F. necrophorum* within batch culture. While *B. licheniformis* has been used as a DFM, current literature demonstrating its effects in beef cattle is limited, and to the authors knowledge it has not been evaluated for its efficacy to decrease liver abscesses.

Growth performance during the receiving period is reported in Table 3. By design, initial BW did not differ ($P = 0.79$) among treatments. In the present study, at the end of the 59-d receiving period, there were no differences in final live BW ($P = 0.25$). Similarly, Opheim (2020), reported no differences in BW among steers fed dietary treatments containing no monensin, tylosin phosphate, or a bacterial DFM containing *Lactobacillus salivarius* after 56 days on feed (DOF**)**. *Lactobacillus salivarius* is characterized as a bacteriocin-producing bacterium commonly isolated from the gastrointestinal tract of humans and animals (Chaves et al., 2017). As a DFM, *L. salivarius* primarily acts by modulating gastrointestinal microbiota, inhibiting colonization by pathogenic bacteria, and modifying fermentation end-

products (Ayala et al., 2017; Chaves et al., 2017). In contrast, Smock et al. (2020) observed an 11 kg difference in final live BW at the end of a 56-d receiving period for steers supplemented with a *Bacillus subtilis*-based DFM versus steers fed a control diet without a DFM. Known as a gram-positive, spore-forming, facultative anaerobe, *B. subtilis* acts by passing through the rumen and initiating activity in the small intestine and lower gastrointestinal tract to inhibit pathogenic organisms and produce antimicrobials for host protection (Lin et al., 2007; Seo et al., 2010). After 56 DOF, increases in final live BW noted by Smock et al. (2020) were likely a result of increases in DMI and ADG seen throughout the receiving period. During the receiving phase, no differences in final live BW were expected, as DMI and ADG did not differ among treatments.

From d 0 to d 30, ADG, DMI, DMI as a percentage of BW, and G:F did not differ ($P \geq 0.21$). Likewise, from d 31 to d 59, ADG, DMI, DMI as a percentage of BW, and G:F did not differ ($P \geq 0.30$). Additionally, in the overall receiving period from d 0 to d 59, there were no differences in ADG, DMI, DMI as a percentage of BW, or G:F ($P \geq 0.20$). Conversely, Opheim (2020) observed a 5.10% decrease in DMI during the first 56 DOF in cattle fed monensin in combination with a bacterial DFM with *L. salivarius* compared to cattle fed a combination of monensin sodium and tylosin phosphate. The lack of difference in DMI during the receiving phase in the present study, indicate that a *B. licheniformis*-based DFM fed in combination with monensin sodium has no deleterious effects on DMI early in the feeding period. Smock et al. (2020) noted increases in DMI on d 14, 28, 56, and overall, from d 0 to 56 when cattle were supplemented with a *B. subtilis*-based DFM versus those fed a diet containing no monensin. Historically, DMI data for cattle supplemented with a DFM is inconsistent (Krehbiel et al., 2003). Differences in DFM bacterial species, or combination of species, have the potential to increase propionate concentration in the rumen and decrease the time spent in subacute acidosis with a decreased ruminal pH (Krehbiel et al., 2003). Therefore, it is likely that DFM bacterial strains and feeding combinations can affect DMI differently.

According to Smock et al. (2020), when cattle were supplemented with a *B. subtilis*-based DFM versus cattle fed a control diet not containing monensin, cattle fed the DFM had greater ADG from d 0 to 14 and over the entire receiving period from d 0 to 56. Opheim (2020) noted no differences in ADG after 56 DOF for cattle fed a bacterial DFM with *L. salivarius*. Similar to the present study, Smock et al. (2020) noted no differences in G:F over the entire receiving period. Wilson et al. (2019), fed the same *B. subtilus*-based DFM and reported a tendency for increased G:F during the receiving period over cattle fed a control diet without the DFM. During the receiving period, no differences in final BW and ADG were noted, therefore, no difference in G:F among treatments was expected.

Growth performance during the finishing period is reported in Table 4. During the finishing period, live- and carcass-adjusted final BW did not differ ($P \geq 0.57$) among treatments. Similar to the present study, when cattle were supplemented with a *B. subtilis*-based DFM, there were no observed differences in final live BW (Wilson et al., 2019). In contrast, a study evaluating the efficacy of a combination bacterial DFM with *Lactobacillus acidophilus* and *Propionibacteria freudenreichii* noted that cattle had greater final live BW than cattle receiving no DFM (Cull et al., 2015). Both *L. acidophilus* and *P. freudenreichii* are gram-positive bacterium that are common inhabitants of the rumen (McAllister et al., 2011). *Lactobacillus acidophilus* and *P. freudenreichii* act by modifying ruminal fermentation end-products to promote the production of propionate and ultimately decrease the risk of subclinical acidosis (Greenquist et al., 2004; McAllister et al., 2011). Opheim (2020) noted a tendency for a decrease in final live BW for cattle fed monensin in combination with a bacterial DFM with *L. salivarius* compared to cattle fed no monensin or a combination of monensin sodium and tylosin phosphate. Nonetheless, no differences were observed in carcass-adjusted final BW, suggesting that the tendency noted in final live BW was because of gut fill (Opheim, 2020).

For the overall finishing period, there were no differences in ADG, DMI, DMI as a percentage of BW, or G:F ($P \geq 0.17$). Likewise, carcass-adjusted ADG, DMI as a percentage of BW, and G:F did not differ ($P \geq 0.16$). Similarly, Huck et al. (2000)

reported when finishing heifers were supplemented with a bacterial DFM containing *L. acidophilus* or *P. freudenreichii*, there were no differences in live- or carcass-adjusted ADG or G:F, and no difference in DMI. Conversely, Opheim (2020) noted a decrease in DMI among steers fed monensin in combination with a bacterial DFM with *L. salivarius,* compared to cattle fed no monensin or a combination of monensin sodium and tylosin phosphate. Additionally, on a live-basis, steers fed a diet containing monensin in combination with a *L. salivarius*-based DFM had decreased G:F and a subsequent decrease in ADG when compared to steers fed no ionophores (Opheim, 2020). Consistent with the present study, no differences were noted in carcass-adjusted G:F or ADG (Opheim, 2020). In studies reviewed by Krehbiel et al. (2003), results suggest that feeding a bacterial DFM to feedlot cattle increases ADG by 2.5 to 5% and increases G:F by approximately 2%. Growth performance differences observed in the present study and published literature represent differences in DFM bacterial strains, feeding combinations, and proposed mechanisms of action. Additionally, the bacterial DFM fed in the present study was selected because it decreases *F. necrophorum* in batch culture and differences in growth performance were not expected.

Carcass characteristics and liver scores are reported in Table 5. Across treatments, no differences in HCW were noted ($P = 0.84$). Dressing percentage, marbling score, longissimus dorsi (LM) area, 12th-rib fat thickness, and calculated yield grade (YG) were not different among dietary treatments ($P \geq 0.32$). Likewise, studies conducted by Brashears et al. (2003) and Vasconcelos et al. (2008) reported no differences in carcass characteristics in cattle fed a DFM with *L. acidophilus* or a combination of *L. acidophilus* and *P. freudenreichii.* Opheim (2020) noted a decrease in marbling score and a numerical decrease in HCW when feeding monensin in combination with a DFM. In contrast, 6 studies reviewed by Krehbiel et al. (2003) summarizing the effects of varying concentrations and strains of *L. acidophilus* and *P. freudenreichii*, reported that cattle fed a bacterial DFM had an increase in HCW when compared to cattle receiving no DFM. In the present study, no difference in HCW was expected as DMI and final live BW did not differ among treatments.

Liver abscess incidence and severity were not affected by dietary treatments ($P \geq 0.13$). Similarly, a study conducted by Luebbe et al. (2013) evaluating the use of 2 commercially available DFM (10-G [*Enterococcus faecium, Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus plantarum* and *Predococcus acidilactici*; Life Products, Inc., Norfolk, NE] and Bovamine® [*Lactobacillus acidophilus* and *Propionibacterium freudenreichii*; Nutritional Physiology Co., Overland Park, KS]) reported no differences in liver abscess incidence between cattle receiving a DFM-based product versus those receiving no DFM. Both commercially available products evaluated by Luebbe et al. (2013) work by competitive exclusion of pathogenic organisms in the lower gastrointestinal tract and alter ruminal fermentation end-products. Mayer et al. (2022) noted no differences in liver abscess incidence when comparing a commercial DFM containing 5 strains of lactic acid producing-bacteria versus a control diet containing no DFM. Although, the incidence of total severe abscesses (A+, A + Open, A + Adhesion, A + Adhesion/Open) was less in cattle fed a diet containing no DFM when compared to cattle receiving a lactic acid bacteria based DFM (Mayer et al., 2022). While no differences in liver abscess severity were noted between dietary treatments in the present study, a numerical difference in severely abscessed livers (A+) was observed between NCON and DFM treatments; 13.3 and 5.36%, respectively. Additionally, when comparing PCON and DFM treatments, total severely abscessed livers did not differ (5.36%), suggesting that a *B. licheniformis*-based DFM could decrease the frequency of severe liver abscesses similar to tylosin phosphate while decreasing the use of antimicrobials during the feedlot production phase.

Among steers fed PCON and PCONW treatments, no difference exists in liver abscess incidence. Likewise, other studies have evaluated the removal or intermittent feeding of tylosin phosphate and its effect on liver abscess incidence and prevalence (Sides et al., 2009; Bohrer et al., 2016; Müller et al., 2018). Sides et al. (2009) noted that withdrawing tylosin from the diet the last 33 to 35 d on feed decreased liver abscess incidence and percentage of abscesses did not differ from feeding tylosin phosphate continuously throughout the feeding period. Results from the present study,

coupled with data from published literature, suggest that tylosin phosphate has the most influential affect in preventing liver abscesses during the feedlot phase. Likewise, tylosin phosphate has a prolonged effect on decreasing liver abscesses even after removal from the diet. Differences in DFM composition from published literature compared to the present study could explain the variations noted in growth performance, carcass characteristics, and liver abscess incidence. The inclusion of a novel direct-fed microbial in finishing cattle diets did not affect growth performance, or carcass characteristics. Additionally, the inclusion of a novel direct-fed microbial did not decrease the percentage of liver abscesses, and therefore is not a viable alternative to tylosin phosphate. Given the limited existing literature in beef cattle, further research evaluating the supplementation of *B. licheniformis* to decrease liver abscesses is warranted.

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TABLES

Table 1. Ingredient and nutrient composition of receiving and transition diets where inclusion of a novel direct-fed microbial or tylosin phosphate differed[1]

	Diets		
Item	Receiving	Transition 1	Transition 2
Ingredient, % DM			
Steam-flaked corn	21.15	35.73	50.05
Ground corn	0.52	0.51	0.49
Sweet Bran[2]	52.38	39.97	30.41
Chopped alfalfa hay[3]	19.96	15.71	10.59
Yellow grease	1.85	3.50	3.34
Limestone	2.13	2.01	2.00
Urea	-	-	0.66
Supplement[4]	2.01	2.57	2.46
Analyzed Composition[5]			
Diet DM, %	69.00	74.10	75.00
Crude protein, %	18.40	14.60	16.20
Neutral detergent fiber, %	34.30	24.20	22.90
Acid detergent fiber, %	14.70	10.40	9.80
Ether extract, %	4.50	6.10	6.20
Ca, %	0.78	0.94	0.99
P, %	0.68	0.52	0.48
NEm[6], Mcal/kg	2.01	2.12	2.18
NEg[6], Mcal/kg	1.36	1.45	1.50

[1]Dry matter basis, except DM %.
[2]Sweet Bran was supplied by Cargill Corn Milling, Blair, NE.
[3]Chopped to a length of 2.5 to 5.1 cm.
[4]Supplement supplied 5.99% potassium chloride, 44.40% crude protein, 3.82% sodium, 8.34 mg/kg cobalt carbonate, 395.00 mg/kg copper sulfate, 408.00 mg/kg iron sulfate, 764 mg/kg manganous oxide, 2.92 mg/kg selenium, 2,490.00 mg/kg zinc sulfate, with each animal consuming an average of 232 mg/steer daily monensin sodium (Rumensin-90; Elanco Animal Health, Greenfield, IN), 78.0 mg/steer daily tylosin phosphate (Tylan-100; Elanco Animal Health, Greenfield, IN), and was formulated to include 89.8 IU/kg vitamin A and 712,000 IU/kg vitamin E on a DM basis. Negative control (NCON) and direct-fed microbial (DFM) dietary treatments did not contain tylosin phosphate.
[5]Analysis performed by ServiTech Laboratories (Amarillo, TX). Actual diet formulation based on weekly DM determinations.
[6]NE_m and NE_g reported as tabular values based on NASEM (2016).

Table 2. Ingredient and nutrient composition of finishing diets where inclusion of a novel direct-fed microbial or tylosin phosphate differed[1]

Item	Finishing Diet	Finishing Diet (0% Yellow Grease)[2]
Ingredient, % DM		
Steam-flaked corn	62.99	62.99
Ground corn	0.44	0.44
Sweet Bran[3]	24.10	26.56
Chopped alfalfa hay[4]	6.11	6.11
Yellow grease	2.46	0.00
Limestone	1.47	1.47
Urea	0.49	0.49
Supplement[5]	1.94	1.94
Analyzed Composition[6]		
Diet DM, %	77.20	76.90
Crude protein, %	12.20	13.10
Neutral detergent fiber, %	17.40	19.20
Acid detergent fiber, %	8.00	8.80
Total starch, %	54.30	57.00
Ether extract, %	4.80	3.00
Ca, %	0.38	0.34
P, %	0.37	0.40
NEm[7], Mcal/kg	2.25	2.19
NEg[7], Mcal/kg	1.56	1.51

[1]Dry matter basis, except DM %.
[2]On February 21, 2022 (d 100) yellow grease was removed from the finishing diet and replaced with Sweet Bran to maintain a least diet cost.
[3]Sweet Bran was supplied by Cargill Corn Milling, Blair, NE.
[4]Chopped to a length of 2.5 to 5.1 cm.
[5]Supplement supplied 5.99% potassium chloride, 44.40% crude protein, 3.82% sodium, 8.34 mg/kg cobalt carbonate, 395.00 mg/kg copper sulfate, 408.00 mg/kg iron sulfate, 764 mg/kg manganous oxide, 2.92 mg/kg selenium, 2,490.00 mg/kg zinc sulfate, with each animal consuming 221 mg/steer daily monensin sodium (Rumensin-90; Elanco Animal Health, Greenfield, IN), 68.0 mg/steer daily tylosin phosphate (Tylan-100; Elanco Animal Health, Greenfield, IN), and was formulated to include 89.8 IU/kg vitamin A and 712,000 IU/kg vitamin E on a DM basis. Negative control (NCON) and direct-fed microbial (DFM) dietary treatments did not contain tylosin phosphate.
[6]Analysis performed by ServiTech Laboratories (Amarillo, TX). Actual diet formulation based on weekly DM determinations.
[7]NE_m and NE_g reported as tabular values based on, NASEM (2016).

Table 3. Receiving period growth performance of beef steers fed to evaluate the use of a novel direct-fed microbial to control liver abscesses and decrease antimicrobial use

	Treatment[1]					
Item	NCON	PCON	PCONW	DFM	SEM[2]	*P*-value
n, steers	56	58	60	60	-	-
n, pens	3	3	3	3	-	-
Initial body weight[3], kg	263	263	264	263	11.2	0.79
Final body weight[4], kg	368	357	365	365	10.4	0.25
d 0 to d 30						
ADG, kg	1.39	1.37	1.46	1.42	0.049	0.58
DMI, kg	6.06	5.82	6.16	5.98	0.130	0.25
DMI, % of BW	2.14	2.06	2.16	2.11	0.058	0.21
G:F	0.230	0.235	0.237	0.238	0.0072	0.83
d 31 to d 59						
ADG, kg	2.17	1.82	1.98	2.04	0.118	0.30
DMI, kg	8.14	7.83	8.19	7.90	0.167	0.37
DMI, % of BW	2.42	2.37	2.44	2.36	0.066	0.46
G:F	0.266	0.232	0.242	0.259	0.0150	0.41
Overall d 0 to 59						
ADG, kg	1.77	1.59	1.72	1.73	0.055	0.20
DMI, kg	7.04	6.77	7.12	6.89	0.130	0.21
DMI, % of BW	2.24	2.19	2.27	2.20	0.057	0.30
G:F	0.252	0.235	0.241	0.251	0.0092	0.43

[1]NCON = dietary supplement contained no tylosin phosphate (Tylan-100; Elanco Animal Health, Greenfield, IN); PCON = dietary supplement contained tylosin phosphate; PCONW = tylosin phosphate removed the last 56 d of the feeding period; DFM = novel direct-fed microbial fed at 1 g mixture/animal with 1×10^{11} CFU/g of DFM.
[2]Standard error of least squares mean (n = 3 pens/mean).
[3]Average of BW on d -1 and 0.
[4]BW was shrunk 4%.

Table 4. Finishing period growth performance and carcass-adjusted performance of beef steers fed to evaluate the use of a novel direct-fed microbial to control liver abscesses and decrease antimicrobial use

	Treatment[1]					
Item	NCON	PCON	PCONW	DFM	SEM[2]	*P*-value
n, steers	52	56	59	58	-	-
n, pens	15	15	15	14	-	-
Initial body weight[3], kg	367	359	366	369	11.2	0.74
Final body weight[3], kg	625	618	627	633	13.8	0.57
Overall finishing[4]						
ADG, kg	1.53	1.54	1.55	1.57	0.068	0.84
DMI, kg	9.45	9.15	9.19	9.54	0.291	0.17
DMI, % of BW	1.91	1.87	1.85	1.90	0.032	0.18
G:F	0.163	0.168	0.169	0.165	0.0039	0.45
Carcass-adjusted						
Final BW[5], kg	626	620	628	631	15.3	0.84
ADG, kg	1.45	1.47	1.47	1.47	0.073	0.97
DMI, % of BW	1.91	1.87	1.85	1.90	0.030	0.16
G:F	0.154	0.160	0.159	0.154	0.004	0.30

[1]NCON = dietary supplement contained no tylosin phosphate (Tylan-100; Elanco Animal Health, Greenfield, IN); PCON = dietary supplement contained tylosin phosphate; PCONW = tylosin phosphate removed the last 56 d of the feeding period; DFM = novel direct-fed microbial fed at 1 g mixture/animal with 1×10^{11} CFU/g.

[2]Pooled standard error of least squares mean (n = 14 to 15 pens/mean).

[3]All BW are shrunk 4%.

[4]For the finishing period, blocks 2 to 3 were 162 days on feed, and blocks 1 were on 183 days on feed. Total average days in the finishing period was 173.

[5]Hot carcass weight divided by overall average DP (64.98%).

Table 5. Carcass characteristics and liver scores of beef steers fed to evaluate the use of a novel direct-fed microbial to control liver abscesses and decrease antimicrobial use

	Treatment					
Item	NCON	PCON	PCONW	DFM	SEM	*P*-value
Hot carcass weight, kg	407	403	408	410	9.70	0.84
Dressing percent[2], %	65.10	65.31	64.86	64.67	0.310	0.32
Marbling score[3]	620	643	626	640	17.3	0.75
12th-rib fat thickness, cm	1.70	1.68	1.77	1.67	0.074	0.80
Longissimus dorsi area, cm sq	95.36	96.48	94.50	96.93	1.654	0.49
Calculated YG	3.35	3.25	3.47	3.27	0.124	0.59
EBF[4], %	31.94	31.78	32.44	31.75	0.484	0.73
AFBW[5], kg	597	590	596	607	27.4	0.33
Choice or greater, %	87.8	96.1	91.7	92.9	3.91	0.51
Liver score, %						
Abscessed	20.6	19.0	12.5	24.4	5.18	0.46
A-[6]	7.22	11.9	9.38	14.9	3.86	0.58
A[7]	0.00	1.78	0.00	4.20	1.60	0.21
A+[8]	13.3	5.36	3.13	5.36	3.10	0.13

[1]NCON = dietary supplement contained no tylosin phosphate (Tylan-100; Elanco Animal Health, Greenfield, IN); PCON = dietary supplement contained tylosin phosphate; PCONW = tylosin phosphate removed the last 56 d of the feeding period; DFM = novel direct-fed microbial fed at 1 g mixture/animal with 10^{11} CFU/g.
[2]Calculated as hot carcass weight divided by final shrunk live BW.
[3]Leading digit indicates score and following digits indicate degree of marbling within score; 5 = modest.
[4]Empty body fat, %. Estimated using equations of Guiroy et al. (2001).
[5]Final shrunk BW adjusted to equivalent 28% EBF using equations of Guiroy et al. (2001).
[6]A- = 1 or 2 small abscesses or inactive scars.
[7]A = 2 to 4 small active abscesses (< 6 cm in diameter); minor abscesses.
[8]A+ = 1 or more large abscesses (> 6 cm in diameter); active abscesses, severe abscesses.

CHAPTER IV

EFFECTS OF A NOVEL DIRECT-FED MICROBIAL ON OCCURRENCES OF ANTIMICROBIAL RESISTANCE IN *SALMONELLA ENTERICA*, *ESCHERICHIA COLI*, AND *ENTEROCOCCUS* SPP. MEASURED LONGITUDINALLY FROM FEEDLOT ARRIVAL TO HARVEST IN FINISHING BEEF STEERS

ABSTRACT

The continuous feeding of antimicrobials, such as tylosin phosphate, for the control of liver abscesses is commonplace in the feedlot. A decrease in antimicrobial use in food animal production is desirable, and therefore, interest in direct-fed microbials as an alternative increases. Two-hundred forty Angus beef steers (mean initial BW = 263 kg ± 18.0 kg) were randomly assigned to one of three dietary treatments; negative control, dietary supplement contained no tylosin phosphate (NCON); positive control, dietary supplement contained tylosin phosphate (PCON); or novel direct-fed microbial fed at 1 g mixture/steer with 1×10^{11} CFU/g (DFM). Fecal samples were collected on day 0, 59, 128 and study end (day 221 or 242). Pen and hide swabs were collected two days before harvest and *subiliac* lymph nodes were collected the day of harvest. All targeted bacterial populations differed across time ($p \leq 0.03$), except 128 mg L^{-1} erythromycin resistant *Escherichia coli.* The effect of treatment differed by day for total *Enterococcus* spp. concentrations and were greatest for the DFM treatment on day 128, 221, and 242 ($p \leq 0.01$). No differences in *Salmonella* prevalence amongst pen swabs, hide swabs, or *subiliac* lymph nodes were detected ($p \geq 0.37$). *Salmonella* resistant to tetracyclines or cefotaxime was not detected in feces. Data from this study suggest that the in-feed inclusion of a novel direct-fed microbial is not directly implicated in antimicrobial resistance of feedlot cattle.

INTRODUCTION

Antimicrobial resistance (AMR) is one of the largest global human-health concerns of the 21st century (CDC, 2019a). In human medicine, the misuse and

over-prescription of antimicrobials are primary factors contributing to the increase in AMR gene prevalence (Michael *et al*., 2014). The use of antimicrobials in animal production increases the possibility for the transfer of AMR resistant genes from livestock to the environment, posing a threat for further contributions to AMR in humans (Michael *et al*., 2014). According to Beauchemin *et al*. (2006), direct-fed microbials (DFM) have the potential to prevent disease while decreasing the need for antibiotic use in ruminant production.

For United States cattle feeders, the continuous feeding of antimicrobials, such as tylosin phosphate, is commonplace for decreasing liver abscesses. Classified as a macrolide antimicrobial, tylosin phosphate, inhibits the proliferation of gram-negative bacteria primarily associated with liver abscesses, *Fusobacterium necrophorum* (Nagaraja and Chengappa, 1998). Tylosin phosphate is not approved for use in human medicine, but it is deemed medically important by the Food and Drug Administration (FDA; Schmidt *et al*., 2020). While not linked to a specific AMR pathogen, the inclusion of tylosin phosphate in finishing diets could serve as a possible reservoir for resistant genes.

The primary resistant bacteria of concern include *Salmonella* and *E. coli*. According to the CDC (2019a), the prevalence of resistant *Salmonella* is increasing, decreasing the effectiveness of available antimicrobials to treat *Salmonella* infections. Uncommonly related to illness and infection, *E. coli* is the most common bacterium isolated from ruminants, making it a concern for the spread of AMR (Scallan *et al*., 2011; CDC, 2019a; Haulisah *et al*., 2021). At harvest, fecal and hide prevalence of *E. coli* and *Salmonella* is correlated with carcass contamination (Bell, 1997; Elder *et al*., 2000; McEvoy *et al*., 2000). The colonization of lymphatic tissue by *Salmonella* has presented the potential for bacterial contamination of ground beef (Bailey *et al*., 2017; Webb *et al*., 2017). The objective of the current study was to determine how a novel direct-fed microbial or tylosin phosphate affected populations of *Salmonella enterica, Escherichia coli,* and *Enterococcus* spp. in feedlot cattle, measured longitudinally from feedlot arrival to harvest.

MATERIALS AND METHODS

This experiment was conducted at the Texas Tech University Burnett Center for Beef Cattle Research and Instruction and was approved by the Texas Tech University Animal Care and Use Committee (approval number 20040-04).

Two-hundred forty Angus steers (initial body weight (BW) = 263 ± 18.0 kg) were used. During the receiving period, 4 dietary treatments were arranged as a randomized complete block design with 3 BW blocks and 3 pen replications per treatment (n = 12 pens total). Within BW block, experimental treatments were randomly assigned to pen and included: 1) negative control, diet formulated without tylosin phosphate (NCON); 2) positive control, diet formulated with tylosin phosphate (PCON); 3) novel direct-fed microbial fed at 1 g mixture/animal with 1×10^{11} CFU/g (DFM). The novel DFM contains *Bacillus licheniformis* isolates from rumen batch culture. On day 59, all cattle were sorted to partially slatted concrete pens to begin the finishing period and previous dietary treatments were maintained.

Animal Management

Detailed methods on animal husbandry and management are reported in Hoffman (2022). Upon arrival, cattle were vaccinated, and administered metaphylaxis and an anthelmintic. Cattle were then placed in soil-surfaced pens, and received ad libitum access to water, grass hay, and a receiving diet. On day 0, cattle were sorted by day -1 BW into experimental treatment groups. Cattle were revaccinated on day 30 and implanted twice throughout the study. On day 128, cattle were readministered an anthelmintic to treat internal and external parasites.

All diets were formulated to include 24 g/ton monensin sodium. The PCON treatment diet additionally included 8 g/ton tylosin phosphate. For the DFM treatment, the DFM mixture was included in the diet with a ground corn carrier. The NCON and PCON diets contained ground corn carrier without the DFM mixture.

Fecal Sampling Procedures

Fecal samples were collected following procedures described by Long *et al*. (2022). Samples were collected on days 0, 59, 128 and study end. Fecal samples were collected by removing any present fecal material from the rectum. The sample was

then placed into a clean sealable bag and shipped to the USDA-ARS, U.S. Meat Animal Research Center (USMARC) in Clay Center, NE for microbial analysis.

Fecal Sample Processing for Escherichia coli and Enterococcus spp.

The microbial analysis of fecal samples for *E. coli* was conducted following procedures described by Agga *et al*. (2016) and Long *et al*. (2022). Briefly, 10 g of each sample was placed into a filter bag, mixed with 90 ml of Tryptic Soy Broth (TSB) containing 100 mM potassium phosphate buffer (18 mM KH_2PO^4 and 82 mM K_2HPO^4, pH 7.2; Sigma), and mixed by hand massage. Enumerations were conducted by spiral plating 50 μl serial dilutions from each subsample onto CHROMagar *E. coli*, CHROMagar ECC, or CHROMagar Orientation. Targeted AMR populations were total, tetracycline-resistant (TET^R, 32 mg L^{-1} tetracycline), trimethoprim-sulphamethoxazole-resistant (COT^R, 4 mg L^{-1} trimethoprim and 76 mg L^{-1} sulphamethoxazole) and cefotaxime-resistant (CTX^R, 2 mg L^{-1} cefotaxime) *E. coli*; total, erythromycin-resistant (ERY^R, 8 mg L^{-1} erythromycin) and highly ERY^R (128 mg L^{-1} erythromycin) *Enterococcus.* Prevalence of samples not confirmed enumerable was determined by direct plating 20 μl of each enrichment onto individualized selective media agar plates. Morphologically distinct colonies were then counted for enumeration or considered presumptive positive for prevalence. All presumptive colonies were picked and confirmed by PCR.

Fecal Sample Processing for Salmonella

The microbial analysis of fecal samples for *Salmonella* was conducted following procedures described by Agga *et al*. (2016) and Long *et al*. (2022). Enriched fecal samples from the procedure described earlier were used to determine fecal prevalence of *Salmonella*. Using direct plating, enumeration was conducted, and a WASP Touch was used to spiral plate 50 μl of each fecal sample onto Xylose Lysine Deoxycholate (XLD) agar. Five-hundred μl of phosphate-buffered saline with Tween and 10 μl anti-*Salmonella* magnetic beads were placed into deep-well 96-well blocks. Next, 500 μl of each enriched sample was transferred to individual wells, and beads from each sample mixed for 15 min at room temperature. Immunomagnetic beads were then removed, washed two times in PBS, and eluted into 100 μl of PBS-Tween.

A 50 μl aliquot was then transferred to 5 ml of Rappaport Vassiliadis (RVS) broth and enriched overnight at 42°C. A 10 μl loop of the RVS secondary enrichment was plated onto XLD, XLD-tet (32 mg L^{-1} tetracycline) or XLD-ctx (2 mg L^{-1} cefotaxime) agar, and plates were incubated overnight at 37°C.

Pen Swab and Hide Swab Collection and Processing

At study end (day 221 for block 2 and 3 and day 242 for block 1), pen and hide swabs were collected using a sponge (Nasco Whirl-Pak) pre-wet with 10 ml of TSB. A 1,000 cm^2 section (from the concrete pen surface or directly behind the shoulder of the animal) was scrubbed and samples were transported back to the USDA-ARS laboratory near Lubbock, TX for processing and analysis. At arrival, an additional 90 ml of TSB solution was added to each sample bag, and all samples were incubated at 37°C for 6 h. Sponges were then massaged, and the suspension streaked with a 10 μl loop onto BGA agar containing novobiocin (25 μg mL^{-1}). One ml of each swab suspension was put into a 1:10 dilution of RVS enrichment broth, vortexed, and incubated at 42°C overnight. Enrichments were then streaked with a 10 μl loop onto BGA agar containing 25 μg mL^{-1} of Novobiocin and all plates were incubated at 37°C for 24 h. Phenotypic colony re-streaks were confirmed via latex agglutination (*Salmonella* Latex Kit; Oxoid). Two phenotypic isolates were selected from positive enrichment plates and placed into a 1:10 dilution of glycerol to TSB.

Subiliac Lymph Node Collection and Processing

Trained personnel from Texas Tech University collected and tracked harvest order using animal tag numbers. A sample of 64.7% *subiliac* lymph nodes were collected from block 2 and 3, and 72.0% were collected from block 1 (164 total) to be analyzed for lymph node prevalence of *Salmonella*. Lymph nodes were then transported to the USDA-ARS near Lubbock, TX and stored at 4°C overnight.

Lymph nodes were processed following procedures described by Long *et al.* (2022). The following day, lymph nodes were aseptically denuded, weighed, and sterilized in a boiling water bath. Approximately 25 g of each lymph node was placed into a lateral filtered stomacher bag and pulverized with a rubber mallet (Arthur *et al*., 2008). Using a stomacher machine (Stomacher® 400 Circulator; Seward Laboratory

Systems Inc.), the mixture was homogenized, a 100 μl aliquot collected, and subsequently spiral plated onto BGA agar containing novobiocin (25 μg mL^{-1}). Plates were incubated at 37°C for 24 h before being counted using an automated colony counter. An additional 1 ml of the lymph node homogenate was placed into a 1:10 dilution of RVS enrichment broth, vortexed, and incubated at 42°C overnight. Another 1 ml of the lymph node homogenate was placed into a 1:10 dilution of Tetrathionate Broth with iodine, vortexed, and incubated at 37°C overnight. Enrichments were then streaked with a 10 μl loop onto BGA agar containing novobiocin (25 μg mL^{-1}). The plates were incubated at 37°C for 24 h. Phenotypic colonies were streaked onto fresh agar and confirmed by latex agglutination. Two phenotypic isolates were selected from positive enrichment plates and put into a 1:10 dilution of glycerol to TSB.

Statistical Analysis

Data expressed in colony-forming units (CFU/g) were converted using a log transformation (log_{10} CFU g^{-1} of feces) for bacterial concentration analyses. A lower limit for detection of enumeration was set at 200 CFU g^{-1} or 2.3 log_{10} CFU g^{-1}. For nonenumerable, enriched samples, a value of 0.5 CFU g^{-1} was used for prevalence negative samples and a value of 100 CFU g^{-1} was used for prevalence positive samples. The PROC MIXED procedure of SAS was used for analysis of fecal data. Pen served as the experimental unit with fixed effects of dietary treatment, time, and dietary treatment × time interaction and the random effect of BW block. The Kenward Roger adjustment was used to correct the degrees of freedom for unequal experimental units among treatments. Pen was the subject of the repeated measures and was included to control for any variation that occurred throughout the study. Simple effect least squares means are presented graphically, and a *p*-value of 0.05 was used to determine significance. The PROC GLIMMIX procedure of SAS was used to analyze hide swab, pen swab, and lymph node *Salmonella* prevalence data as binomial proportions.

RESULTS

In the present study, no treatment × day interactions were detected except where noted subsequently ($p = 0.72$).

Total Salmonella Counts and Prevalence from Feces

No differences in dietary treatment were detected for total *Salmonella* concentrations ($p = 0.35$; Figure 1a). Conversely, total *Salmonella* concentrations differed across day ($p = 0.03$). Amongst all days, total fecal *Salmonella* concentrations were least on day 242 ($p = 0.04$) and less on day 128 than day 59 ($p < 0.01$). Total *Salmonella* counts were fewer on day 221 and 242 than day 59 ($p < 0.01$) and were greatest across all days on day 59 ($p < 0.01$). Enriched fecal samples were tested for *Salmonella* resistant to tetracyclines or third generation cephalosporins, however, no resistance to either antibiotic was detected.

No effect of dietary treatment was noted for total *Salmonella* prevalence from fecal samples ($p = 0.26$; Figure 1b). Across day, a tendency for a difference in total fecal *Salmonella* prevalence was detected ($p = 0.07$), however, differences in prevalence were noted between day 0 and 221 ($p = 0.02$) and day 128 and 221 ($p = 0.02$). The greatest prevalence of total fecal *Salmonella* was detected on day 221 ($p < 0.01$).

Prevalence of Salmonella in Pens, on Hides, and Lymph Nodes

No differences were detected for *Salmonella* prevalence in pen swabs collected at study end ($p = 0.37$; Table 1). Likewise, among dietary treatments, *Salmonella* prevalence in hide swabs was not different ($p = 0.70$). Additionally, no differences were detected among dietary treatments for *Salmonella* prevalence in lymph nodes ($p = 0.57$) and prevalence averaged 14.0% (7.10-21.4%).

Total, 8ERY^R, and 128ERY^R Enterococcus spp. counts from fecal samples

A treatment × day interaction was noted for total *Enterococcus* spp. concentrations ($p < 0.01$; Figure 2a). On day 0, total *Enterococcus* spp. $\log_{10}$ CFU counts of all dietary treatments did not differ ($p \geq 0.11$). On day 59, the DFM treatment had greater fecal concentrations of total *Enterococcus* spp. compared to the NCON and PCON treatment ($p \leq 0.02$). Additionally, total *Enterococcus* spp. concentrations were greater for the DFM treatment compared to the NCON and PCON treatment on day 128 ($p \leq 0.01$). Furthermore, on day 221 and 242, fecal

Enterococcus spp. counts for the DFM treatment were greater than the NCON and PCON treatments ($p \leq 0.01$).

There was no effect of dietary treatment noted for fecal concentrations of erythromycin resistant (8ERY^R, 8 mg L^{-1} erythromycin) *Enterococcus* spp. ($p = 0.34$; Figure 2b). Amongst days, differences were detected in the concentrations of 8ERY^R *Enterococcus* spp. from fecal samples ($p < 0.01$). Fecal concentrations were greater on day 221 than day 0, 59, or 128 ($p \leq 0.01$) and tended to be greater on day 242 than day 59 ($p = 0.08$). The greatest concentrations of 8ERY^R *Enterococcus* spp. were noted on day 221 ($p < 0.01$).

No difference in dietary treatment was noted for erythromycin highly-resistant (128ERY^R, 128 mg L^{-1} erythromycin; Figure 2c) *Enterococcus* spp. ($p = 0.17$). For day 0, 128ERY^R *Enterococcus* spp. concentrations were least ($p < 0.01$) and were less for day 59 than day 221 or 242 ($p \leq 0.01$). The greatest fecal concentrations of 128ERY^R *Enterococcus* spp. were noted on day 221 ($p < 0.01$).

Total, TET^R, COT^R, CTX^R, and 128ERY^R Escherichia coli Counts and Prevalence from Feces

A tendency for a treatment × day interaction in mean fecal concentrations for total *E. coli* was noted ($p = 0.10$; Figure 3a) and no further effect of dietary treatment was detected ($p = 0.22$). Across day, differences in total *E. coli* concentrations were noted ($p < 0.01$). On day 0, total fecal *E. coli* concentrations were least ($p < 0.01$) and were less on day 128 than day 221 or 242 ($p \leq 0.01$). Total *E. coli* counts were greatest amongst all days on day 221 ($p < 0.01$).

No differences in fecal concentrations of tetracycline resistant (TET^R, 32 mg L^{-1} tetracycline) *E. coli*, ($p = 0.27$; Figure 3b) or effect of dietary treatment was detected ($p = 0.81$). However, differences in fecal concentrations among days were noted ($p < 0.01$). The TET^R *E. coli* fecal concentrations were least ($p < 0.01$) on day 128 and were less on day 242 than day 221 ($p = 0.04$). Amongst all days, day 221 TET^R *E. coli* concentrations were greatest ($p < 0.01$).

No effect of dietary treatments was noted for trimethoprim-sulfamethoxazole resistant (COT^R, 76 mg L^{-1} sulfamethoxazole and 4 mg L^{-1} trimethoprim) *E. coli* ($p =$

0.99; Figure 4a). Across day, differences in COT^R *E. coli* concentrations were noted ($p < 0.01$). On day 0, fecal concentrations of COT^R *E. coli* were least ($p < 0.01$). On day 128, the COT^R *E. coli* concentrations of fecal samples were less than on day 59, 221, or 242 ($p \leq 0.05$) and were greatest amongst all days on day 242 ($p < 0.01$).

For the fecal prevalence of COT^R *E. coli,* no differences in dietary treatment were noted ($p = 0.96$; Figure 4b). However, differences in percent prevalence of COT^R *E. coli* were detected across day ($p < 0.01$). On day 0, the prevalence of COT^R *E. coli* was least when compared amongst days ($p < 0.01$). A tendency for percent prevalence was noted between day 59 and 128 ($p = 0.07$) and day 128 and 221 ($p = 0.06$), with the greatest prevalence on day 59 ($p < 0.01$). Additional differences in COT^R *E. coli* prevalence were noted between day 128 and 242 ($p < 0.01$).

No effect of dietary treatment was detected in fecal concentrations of cefotaxime resistant (CTX^R, 2 mg L^{-1} cefotaxime) *E. coli* ($p = 0.92$; Figure 5a). Across day, differences in the concentrations of CTX^R *E. coli* were noted ($p < 0.01$). On day 128, the fecal concentrations of CTX^R *E. coli* were least ($p < 0.01$) and less on day 0 than day 221 or 242 ($p \leq 0.01$). On day 242, fecal concentrations of CTX^R *E. coli* were greatest ($p < 0.01$).

For the percent prevalence of CTX^R *E. coli*, no effect of dietary treatment was noted ($p = 0.58$; Figure 5b). However, differences in prevalence across day were detected ($p < 0.01$). Percent prevalence of CTX^R *E. coli* was least on day 0 ($p < 0.01$) and greatest amongst all days on day 59 ($p < 0.01$). On day 128, prevalence of CTX^R *E. coli* was less than on day 59, 221, or 242 ($p \leq 0.07$).

For erythromycin resistant (ERY^R, 128 mg L^{-1} erythromycin) *E. coli*, no effect of dietary treatment ($p = 0.21$; Figure 5c) or effect of day ($p = 0.47$) was detected.

DISCUSSION

In the present study, NARMS antibiotic resistance surveillance recommendations were used to evaluate antimicrobial resistance targets and concentrations.

Salmonella

Annually, the CDC estimates about 1.35 million infections, 26,500 hospitalizations, and 420 deaths are caused by *Salmonella* bacteria (CDC, 2022). However, an increase in multidrug-resistant *Salmonella* poses an increasing health risk to humans (Varma *et al*., 2005). Implementation of antimicrobial interventions by commercial beef processing facilities have been effective in reducing *Salmonella* prevalence on hides and carcasses at harvest (Arthur *et al*., 2007; Arthur *et al*., 2008). While many intervention strategies aim to decrease surface-level contamination of carcasses, colonization of the lymphatic system by *Salmonella* creates the potential for contamination of ground beef products (Gragg *et al*., 2013; Li *et al*., 2015).

In the current study, differences in total *Salmonella* concentrations across day were detected and a tendency for percent prevalence over time was noted; however, no differences in fecal concentrations or prevalence were noted among dietary treatments. Over time, total fecal *Salmonella* concentrations generally decreased in the current experiment. Conversely, Levent *et al*. (2019) reported that fecal *Salmonella* concentrations and percent prevalence increased on days 7, 14, 28, and 56 in beef cattle administered either tulathromycin or ceftiofur. In a study by Long *et al*. (2022) longitudinally measuring changes in *Salmonella* for cattle administered metaphylaxis with tulathromycin, ceftiofur, or florfenicol, over 242 or 252 days, increases in fecal concentration and percent prevalence were noted. In the present study on day 59, all cattle were sorted to partially slatted concrete-surfaced pens from soil-surfaced pens to begin the finishing period. Subsequently, after day 59, total fecal *Salmonella* concentrations were decreased, suggesting a decrease in the survival rate of *Salmonella* on concrete compared to soil. Additionally, the USDA NAHMS 1999 feedlot survey, reports that *Salmonella* populations increase as ambient temperatures increase across seasons. Barkocy-Gallagher *et al*. (2003) sampled cattle carcasses from three commercial abattoirs throughout the Midwest by season and reported that *Salmonella* prevalence was greatest in the summer months when compared to colder months (winter and spring). Similarly, Brichta-Harhay *et al*. (2011) noted that seasonal diversity in *Salmonella* was lowest in the winter and greatest in the fall among samples obtained every 3 months in a 10-month period from commercial

abattoirs located in the Midwest. Results from the present study contradict these findings, as the greatest $\log_{10}$ CFU counts of *Salmonella* were noted on day 59 (January 11, 2022). Differences in geographical location and changes in environment from a soil-surfaced pen to a concrete-surface pen could explain the lack of seasonal diversity in *Salmonella* concentrations seen in the present study.

The average prevalence of *Salmonella* from *subiliac* lymph nodes were not different among dietary treatments. In a study conducted at the same experimental location, Long *et al*. (2022) noted average prevalence of *Salmonella* from hide swabs was 55.4% when comparing cattle exposed to a metaphylactic antimicrobial versus no antimicrobial exposure; a 43.6% increase when compared to the current study. Furthermore, Long *et al*. (2022) noted average prevalence of *Salmonella* from lymph nodes was 8.40% after 242 or 252 days; a 5.60% decrease in comparison to the present experiment. Conversely, Levent *et al*. (2019) reported that cattle given a metaphylactic antimicrobial with tulathromycin or ceftiofur had no differences in *Salmonella* prevalence amongst hide swabs or lymph nodes after 99, 120, 134, or 141 days. Differences observed by Long *et al*. (2022), Levent *et al*. (2019), and the present study suggest that differences in antimicrobial route of administration, type of antimicrobial, and time, likely altered observed *Salmonella* concentrations. Webb *et al*. (2017) suggested that feedlot cattle possess a greater prevalence of *Salmonella* in lymph nodes during warmer months when compared to cooler months, 11.6 and 2.7% respectively. In the present experiment, cattle were harvested during summer months (June and July) and differences in percent prevalence of *Salmonella* isolated from *subiliac* lymph nodes were not detected. Likewise, Long *et al*. (2022) reported no differences in *Salmonella* percent prevalence of *subiliac* lymph nodes from cattle harvested during summer months.

From pen swabs and hide swabs, the average prevalence of *Salmonella* among dietary treatments was not different. The cross-contamination of cattle hides often occurs because of direct contact with the feedlot pen floor surface. Therefore, because pen swab *Salmonella* percent prevalence was not different among treatments, no differences in hide swab *Salmonella* prevalence were expected. Findings from the

present experiment suggest that the inclusion of a novel DFM or tylosin phosphate in finishing diets, minimally contributed to the bacterial prevalence of *Salmonella* in pen swabs, hide swabs, or isolated from *subiliac* lymph nodes.

Enterococcus spp.

Similar to Long *et al*. (2022), *Enterococcus* spp. was monitored for potential pathogenic Gram-positive bacteria because of its recognition by NARMS as a broadly distributed indicator to track AMR in Gram-positive species (FDA, 2020). *Enterococcus* are gram-positive, commensal bacteria of the human and bovine gastrointestinal tracts and are often associated with life-threatening infections (Heuer *et al*., 2006). *Enterococcus hirae* is the predominant spp. isolated from bovine feces and is not commonly associated with infections in human medicine (Anderson *et al*., 2008).

Because of its classification by the World Health Organization (WHO) as critically important, macrolide antimicrobials, such as tylosin phosphate, require risk-management strategies to decrease the threat of AMR (Collignon *et al*., 2009). While tylosin phosphate is not used in human medicine and not linked to a specific AMR pathogen, bacteria resistant to tylosin can cross-select for resistance to other antimicrobials such as erythromycin, a macrolide widely used to treat human infection (Roberts, 2008; Schmidt *et al*., 2020). Jacob *et al*. (2008) reported that *Enterococcus* isolates from cattle fed monensin or a combination of monensin and tylosin phosphate, were more resistant to erythromycin and tylosin compared to isolates from cattle fed no antimicrobials. Furthermore, research conducted by Zaheer *et al*. (2013) reported that the subtherapeutic or therapeutic administration of a macrolide increased the proportion of erythromycin resistant *Enterococcus hirae.* Additionally, Schmidt *et al.* (2020) reported that the in-feed inclusion of tylosin phosphate subsequently increased the proportion of macrolide resistant *Enterococcus*.

In the present study, fecal concentrations of total *Enterococcus* differed among treatments on specific days. These results suggest that exposure to a novel DFM or tylosin phosphate over time, influences the total concentration of *Enterococcus*. Commonly included in livestock production, ionophores, such as monensin sodium,

are antimicrobials used to increase the efficiency of growth; however, because of their proposed mechanism of action, they are effective against gram-positive bacteria (Callaway *et al*., 2003; Russell and Houlihan, 2003). Therefore, the inclusion of monensin in finishing cattle diets likely altered the prevalence of *Enterococcus*. Because cattle receiving the novel DFM additionally received monensin, it is possible that a synergistic relationship existed between the DFM and *Enterococcus* and that monensin had minimal effect on the novel DFM. The potential synergistic relationship subsequently allowed for greater fecal concentrations of *Enterococcus* on day 128, 221, and 242, among the DFM treatment when compared to the negative control and tylosin phosphate treatment. Similarly, Long *et al*. (2022) noted increases in total fecal *Enterococcus* concentrations among treatments over time when cattle were fed monensin. Beukers *et al.* (2015) and Schmidt *et al.* (2020), reported that the exposure of cattle to in-feed tylosin phosphate compared to cattle not receiving tylosin phosphate, increased the concentration of *Enterococcus* over time when monitored over 197 or 231 days.

Differences in the concentration of $8ERY^R$ and $128ERY^R$ *Enterococcus* over time were noted in the current experiment. Fecal concentrations of $8ERY^R$ and $128ERY^R$ *Enterococcus* were greatest on day 221. Dietary treatments did not affect $\log_{10}$ CFU counts of $8ERY^R$ or $128ERY^R$ *Enterococcus*, suggesting the inclusion of a novel DFM or tylosin phosphate minimally contributed to the presence of AMR *Enterococcus* in the current experiment. Similarly, Müller *et al*. (2018) reported that among dietary treatments with varying feeding strategies of tylosin phosphate, differences in resistant *Enterococcus* concentrations were not reported; however, as time in the feeding pen increased, concentrations of erythromycin and tetracycline resistant *Enterococcus* increased. Results from the current study suggest that the in-feed inclusion of a novel DFM could contribute to increases in total fecal *Enterococcus* concentrations over time; however, minimally contributed to the overall presence of AMR *Enterococus* in feces.

Escherichia coli

Within the feedlot, fecal *E. coli* prevalence has the potential to impact hide and carcass contamination at harvest (Elder *et al*., 2000; McEvoy *et al*., 2000; Omisakin *et al*., 2003). Within the digestive tract, *E. coli* has been implicated as a reservoir for antimicrobial resistant genes and readily exchanges genetic material with other bacterial species (Hartl and Dykhuizen, 1984; Blake *et al*., 2003). Therefore, *E. coli* may pass antibiotic resistant genes to transient bacterial pathogens that cause infections in human medicine (Hummel *et al.*, 1986).

In the present study, differences in total, TET, COT, and CTX resistant *E. coli* concentrations and percent prevalence were noted across time. However, dietary treatments did not affect $\log_{10}$ CFU counts or prevalence. Differences in 128ERY^{R} *E. coli* were not noted across time or among dietary treatments. Generally, the prevalence and concentrations of total, COT^{R}, and CTX^{R} *E. coli* increased over time; however, decreases in $\log_{10}$ CFU counts on day 128 were noted for total and all AMR *E. coli* fecal concentrations. Furthermore, changes in fecal concentrations at day 128 were likely affected by seasonal changes. Around day 128 (March 21, 2022), a significant decrease in temperature occurred (-17°C), suggesting the decreased concentrations of total and AMR *E.coli* seen in the current experiment. A study by Long et al. (2022) reported similar trends, in which total *E. coli* concentrations decreased following a significant cold weather event. Likewise, Vikram *et al*. (2017), reported that during colder months, there was less prevalence of generic, TET, COT, and CTX resistant *E. coli*, than in warmer months.

While dietary treatments in the current study did not affect fecal concentrations or prevalence of *E. coli*, current literature demonstrates the potential for DFM to decrease the prevalence of *E. coli* shed in beef cattle feces. Brashears *et al*. (2003), reported that cattle fed a *Lactobacillus acidophilus*-based DFM for approximately 60 days pre-harvest, were 50% less likely to shed *E. coli* when compared to cattle not receiving a DFM. In another study by Arthur *et al.* (2010) evaluating the use of a *Bacillus subtilis*-based DFM, there were no reported differences in fecal *E. coli* prevalence from cattle supplemented the DFM versus those not receiving DFM supplementation. Furthermore, Tabe *et al*. (2008) reported that cattle supplemented

with a combination bacterial DFM (*L. acidophilus* and *Propionibacterium freudenreichii*) were three times less likely to shed *E. coli* in their feces compared to cattle not receiving a DFM. Current literature demonstrating the effects of *B. licheniformis* as a DFM in beef cattle is limited and to the authors knowledge has not been evaluated for its efficacy to decrease fecal shedding of *E. coli*.

CONCLUSIONS

In conclusion, decreases in total fecal concentrations of *Salmonella* and TET^R *E. coli* were noted over the feeding period. The in-feed inclusion of a novel DFM increased total fecal concentrations of *Enterococcus* compared to NCON and PCON treatments. Increases in fecal concentrations of $8ERY^R$ and $128ERY^R$ *Enterococcus*, total *E. coli*, and COT^R and CTX^R *E. coli* were noted over the feeding period and generally increased as days on feed increased. No differences for *Salmonella* prevalence were noted among dietary treatments. The inclusion of a novel DFM or tylosin phosphate is not directly implicated in AMR in feedlot cattle. Further research to examine the efficacy of DFM and their effect on AMR is warranted.

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TABLES AND FIGURES

Table 1. *Salmonella* prevalence in pens, on hides, and lymph nodes of beef steers fed to evaluate the use of a novel direct-fed microbial to control liver abscesses and decrease antimicrobial use after 221 or 242 d

	Treatment[1]				
Item	NCON	PCON	DFM	SEM[2]	*P*-value
n, pens	15	14	14	-	-
Pen *Salmonella* prevalence, %	26.62	22.84	38.03	10.4	0.37
Hide *Salmonella* prevalence, %	14.44	7.92	12.94	5.99	0.70
Subiliac lymph node *Salmonella* prevalence, %	13.33	7.14	21.43	9.15	0.57

[1]NCON = dietary supplement contained no tylosin phosphate (Tylan-100; Elanco Animal Health, Greenfield, IN); PCON = dietary supplement contained tylosin phosphate; DFM = novel direct-fed microbial fed at 1 g mixture/animal with 1×10^{11} CFU/g of DFM.
[2]Pooled standard error of least squares mean (*n* = 14 to 15 pens/mean).

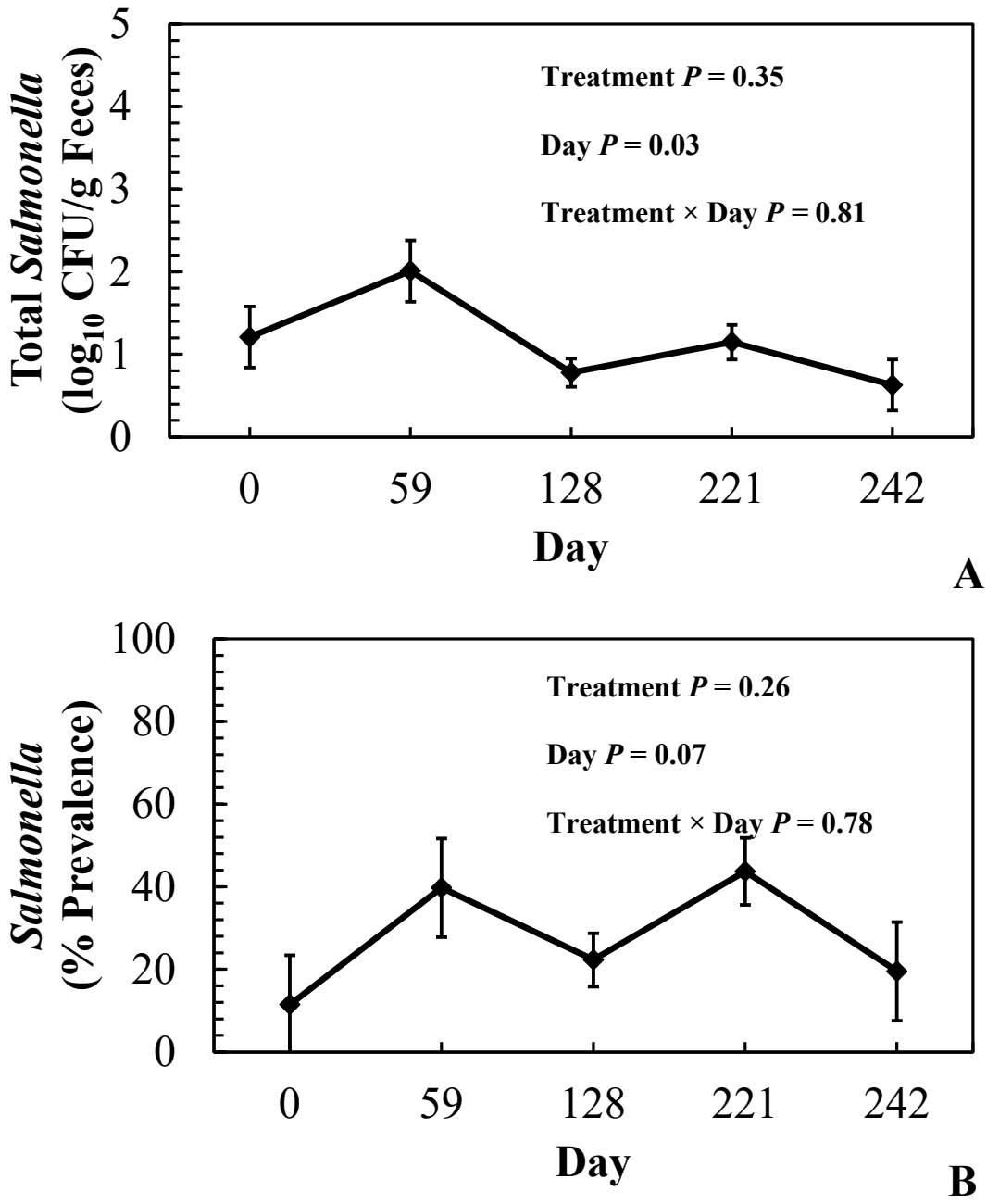

Figure 1. A. Log_{10} counts·g^{-1} feces of *Salmonella* plated on agar without antimicrobial supplementation and **B.** Percent prevalence of *Salmonella* plated on agar without antimicrobial supplementation in fecal samples collected from cattle fed to evaluate the use of a novel direct-fed microbial compared to cattle fed no direct-fed microbial or tylosin phosphate.

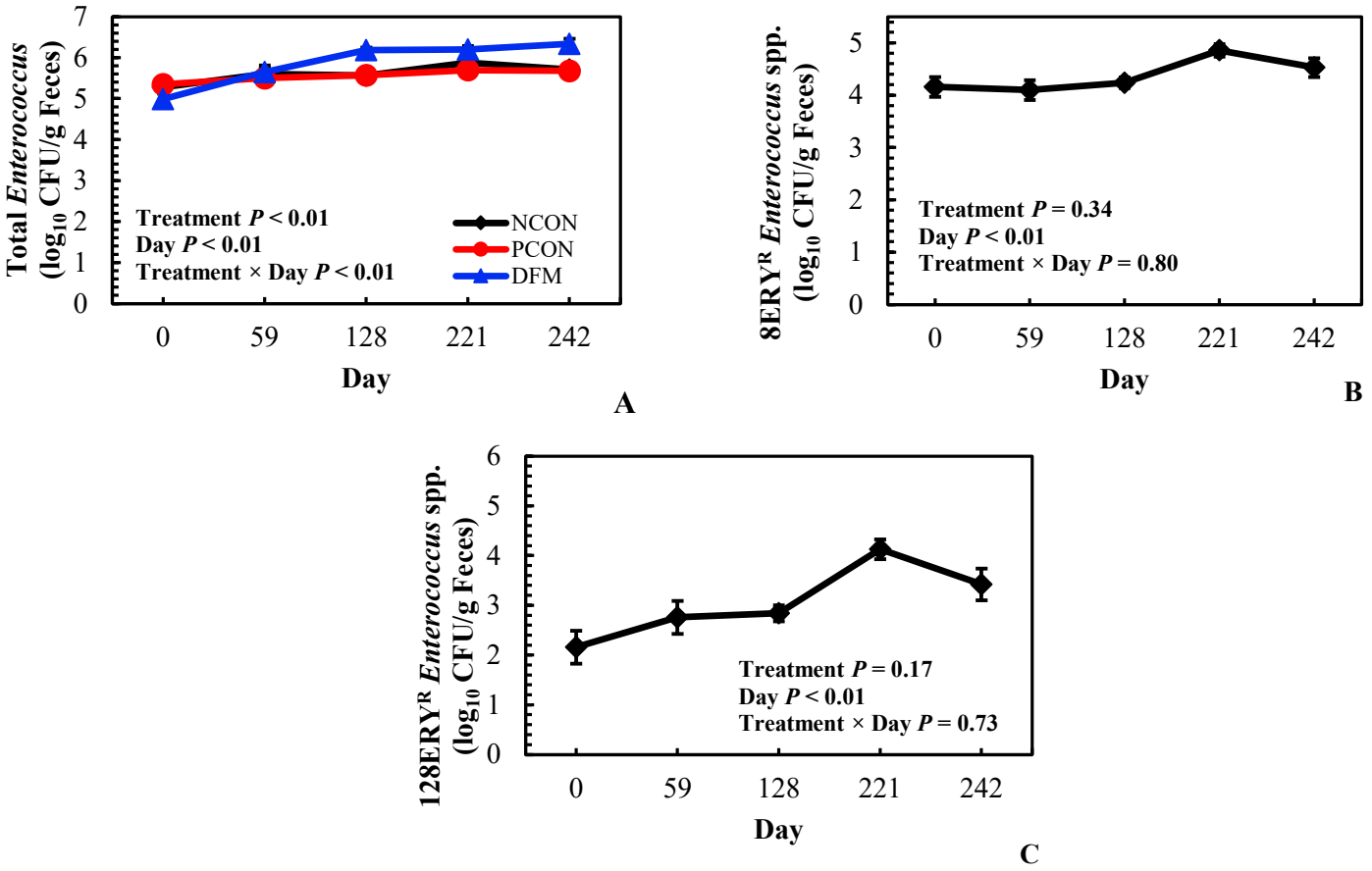

Figure 2. A. Log_{10} CFU counts·g^{-1} feces of *Enterococcus* spp. plated on agar without antimicrobial supplementation from cattle fed to evaluate the use of a novel direct-fed microbial (DFM; blue line; ▲) or tylosin phosphate (PCON; red line; ●) compared to cattle receiving no direct-fed microbial or tylosin phosphate (NCON; black line; ♦), **B.** Erythromycin resistant ($8ERY^R$, 8 mg·L^{-1} erythromycin) *Enterococcus* spp., and **C.** Erythromycin resistant ($128ERY^R$, 128 mg·L^{-1} erythromycin) *Enterococcus* spp. in fecal samples.

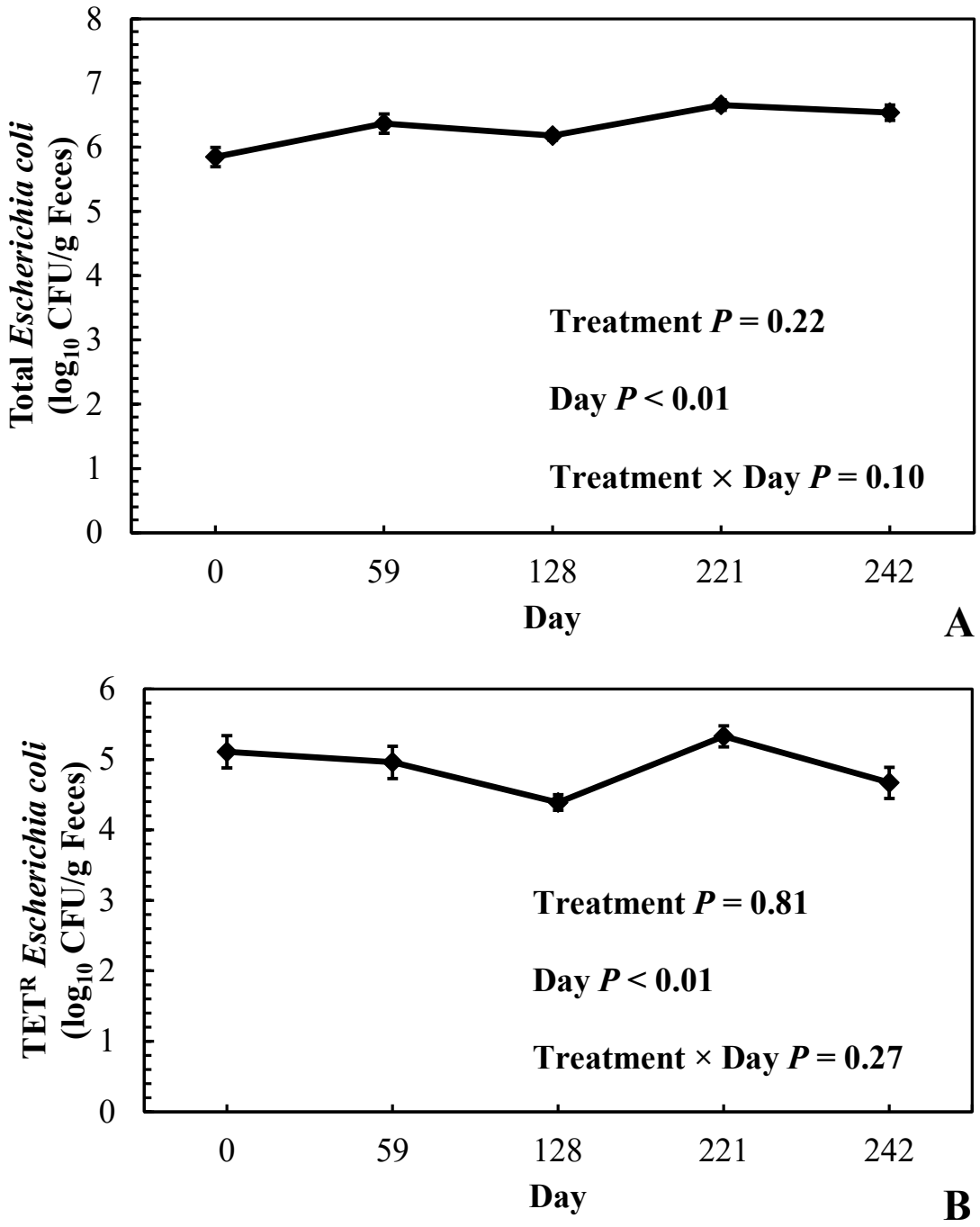

Figure 3. A. Log_{10} CFU counts·g^{-1} feces of Escherichia coli plated on agar without antimicrobial supplementation and **B.** Tetracycline resistant (TET^R, 32 mg·L^{-1} tetracycline) Escherichia coli in fecal samples collected from cattle fed to evaluate the use of a novel direct-fed microbial compared to cattle fed no direct-fed microbial or tylosin phosphate.

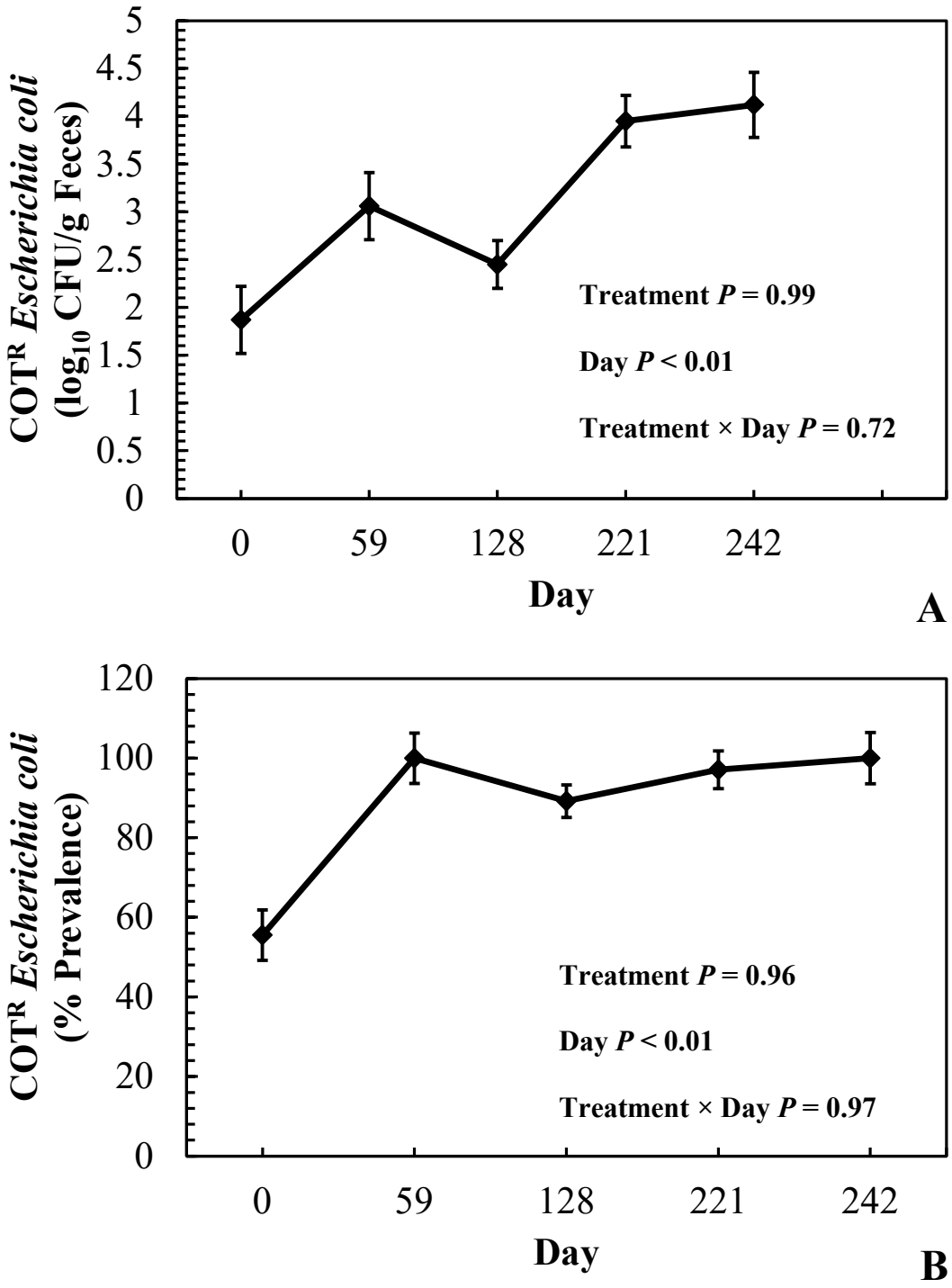

Figure 4. A. Log_{10} CFU counts·g^{-1} feces of trimethoprim-sulfamethoxazole resistant (COT^R, 76 mg·L^{-1} sulfamethoxazole and 4 mg·L^{-1} trimethoprim) Escherichia coli and **B.** Percent prevalence of trimethoprim-sulfamethoxazole resistant (COT^R, 76 mg·L^{-1} sulfamethoxazole and 4 mg·L^{-1} trimethoprim) Escherichia coli in fecal samples collected from cattle fed to evaluate the use of a novel direct-fed microbial compared to cattle fed no direct-fed microbial or tylosin phosphate.

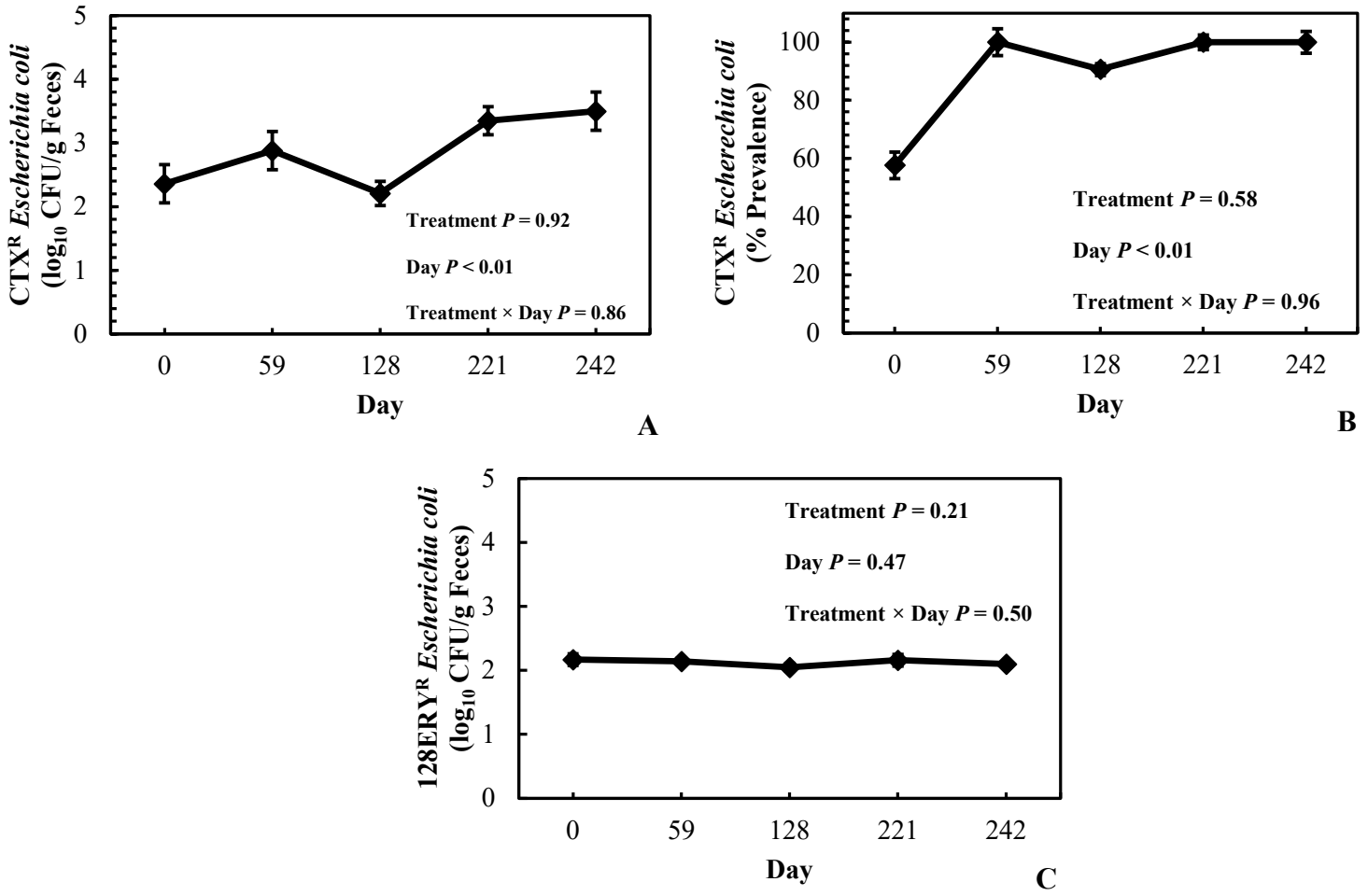

Figure 5. A. Log_{10} CFU counts·g^{-1} feces of 3rd generation cephalosporin resistant (CTX^R, 2 mg·L^{-1} cefotaxime) Escherichia coli, **B.** Percent prevalence of 3rd generation cephalosporin resistant (CTX^R, 2 mg L^{-1} cefotaxime) Escherichia coli, and **C.** Concentration of erythromycin resistant (ERY^R, 128 mg·L^{-1} erythromycin) Escherichia coli in faecal samples collected from

cattle fed to evaluate the use of a novel direct-fed microbial compared to cattle fed no direct-fed microbial or tylosin phosphate.